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Spice-Cured Sprats Ripening, Sensory Parameters Development, and Quality Indicators

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LOODUS - JA TÄPPISTEADUSED B141

Vürtsikilu valmimine, sensoorsete omaduste kujunemine ja kvaliteediindikaatorid

LOREIDA TIMBERG

TTÜ KIRJASTUS

CONTENTS

ABSTRACT

Spice-cured sprat is a traditional culinary product in Estonia and other Eastern-European countries. Spice-cured sprats are made from baltic sprat (*Sprattus sprattus balticus*). Baltic sprat is a nutritionally valuable and delicious fish, however, due to its small size (length ~12 cm, weight ~12 g) its resources are incompletely exploited in food purposes. The purpose of this work is to study the dynamics of chemical, physical, microbiological, and sensory properties of spice-cured sprats during ripening, and evaluate the effect of freezing-thawing and packaging conditions in this process.

Raw material quality is the most important prerequisite when preparing high quality food products. In spice-cured sprats, lipid content is an important parameter in creating sensory perception and a nice full and succulent mouthfeel. In addition, sprat fat content correlates with intestinal enzymatic activities. A descriptive sensory analysis method for baltic sprat was created and results of this survey are related to compositional analysis. In the autumn (September – November) and winter (December – February) periods, sprat samples are harder, sweeter and have a higher lipid content than spring (March – May) and summer (June – August) sprats. These groups suggest that baltic sprats in different catches during the year do have distinct sensory properties, which can influence product quality.

Spice-cured sprat ripening was described by measuring water content, pH, free amino acids, viscoelastic, and sensory properties. In the study a descriptive sensory analysis method for spice-cured sprats was created, where 2 attributes for appearance, 3 attributes for texture, and 17 attributes for flavor were analyzed. The most important attributes which set apart spice-cured sprats *via* ripening time were: hardness, humidity, general spiciness, sour taste, and rancid flavor. Free amino acid content increased and basic/acidic amino acid ratio (B/A) of spice-cured sprats decreased through ripening. The desired sensory properties were obtained while B/A was between 1.0 and 1.5. When comparing sensory properties, the hardness of the spice-cured sprats had decreased and the moistness had increased to a point where a nice succulent mouthfeel was achieved, and where the general spiciness of fish was highest. At this point sour and rancid notes did not dominate.

The effects of freezing-thawing on fish quality in the curing process were reflected by a slower rate of free amino acid formation and higher hardness. In spice-cured samples made from frozen-thawed fish, sour and rancid tastes developed more rapidly than in samples made from fresh fish.

Amino acid formation in plastic jars is higher than in glass jars. At week 8, we observed lower hardness, and higher sourness and rancidness than spice-cured sprats in glass jars. This is related to higher microbial activity due to a better availability of oxygen.

In spice-cured sprats, three species having plate counts over 10^6 CFU·g^{-1} were detected: *Brochothrix thermospacta*, *Lactobacillus sakei*, and *Aerococcus viridians*. Different bacteria were found to dominate between different samples and experimental points. This is ex-

plained by microbial heterogeneity of the sprat microbiota. *Aerococcus viridians* was not detected in spice-cured sprats made from frozen-thawed fish, suggesting the negative effect of freezing on the cultivability and viability of those bacteria. *Lactobacillus sakei* plate counts increased in spice-cured sprats with increasing storage time. This study supports the view that this is a spoilage effect and does not improve the ripening of *Lactobacillus sakei*.

A comparative acceptance study of spice-cured sprat products in Estonia and Thailand shows that products scored lower in the new market, which is expected. However, the consumer acceptance study indicated that spice-cured sprat products in general can be accepted by Thai consumers, especially as part of meals, if additional flavor development is promoted in the products (decrease in salt and increase in acidity).

KOKKUVÕTE

Eestis ja Ida-Euroopas on vürtsikilu olnud traditsiooniline ja armastatud toode juba üle saja aasta. Vürtsikilu valmistatakse läänemere kilust (*Sprattus sprattus balticus*). Läänemere kilu on maitsev ja kõrge toiteväärtusega kala, kuid tema piiratud kasutamine inimtoiduks on tingitud kala väikesest suurusest (pikkus ~12 cm, kaal ~12 g). Käesoleva töö eesmärk oli kirjeldada vürtsikilu valmimise keemiliste, füüsikaliste, mikrobioloogiliste ja sensoorsete omaduste dünaamikat, ja hinnata külmutamise-sulatamise ja erinevate pakkematerjalide mõju sellele protsessile.

Toormaterjali kvaliteet on määravaim tegur heade ja stabiilsete maitseomadustega toidutoodete tootmisel. Vürtsikilu puhul on üheks tähtsamaks toormaterjali kvaliteedi omaduseks kala rasvasisaldus, mis mõjutab tootele iseloomulike organoleptiliste parameetrite kujunemist ja on eriti oluline suussulava tekstuuri ja täidlase suutunde kujunemisel. Lisaks on teada, et kilu rasvasisaldus korreleerub ensümaatilise aktiivsusega, mis on üks olulisemaid tegureid vürtsikilu valmimisprotsessis. Kilu organoleptiliste omaduste hindamiseks loodi sensoorne kirjeldav meetod ja saadud tulemused analüüsiti koos kilu koostise andmetega, mille tulemused olid järgnevad: sügise (september – november) ja talve (detsember – veebruar) kiluproovid olid kõvemad, magusamad ja kõrgema rasvasisaldusega kui kevade (märts – mai) ja suve (juuni – august) kiluproovid. Selline kiluproovide grupeerumine näitab, et kilu organoleptilised omadused on püügihooajati erinevad ja see omakorda võib mõjutada toodete kvaliteeti.

Vürtsikilu valmimisprotsessi kirjeldati veesisalduse, pH, vabade aminohapete, reoloogiliste ja organoleptiliste omaduste kaudu. Vürtsikilu valmimine kaardistati sensoorse kirjeldava meetodi abil, kus hinnati kaht välimuse omadust, kolme tekstuuri omadust ja 17 maitse ja lõhna omadust. Olulisemad organoleptilised omadused, mis eristasid vürtsikiluproove valmimisajas olid: kõvadus, niiskus, üldine vürtsisus, hapu maitse ja rääsunud kõrvalmaitse. Vabade aminohapete sisaldus kasvas ja aluseliste/happeliste aminohapete suhe (A/H) vähenes valmimisaja jooksul ning vürtsikilule iseloomulik sensoorne profiil saavutati kui A/H väärtus oli vahemikus 1.0 – 1.5. Selleks katsepunktiks oli organoleptilistest omadustest vähenenud kõvadus ning kasvanud niiskus ja vürtsikilu oli muutunud optimaalselt pehmeks ja suussulavaks. Samuti oli üldine vürtsisus kõrgeim ning hapu ja rääsunud maitse ei olnud veel kujunenud tajutavaks ning domineerivaks.

Kala külmutamise-sulatamise mõju hindamine vürtsikilu valmimisprotsessile näitas, et vabad aminohapped moodustusid aeglasemalt ja kõvadus oli kõrgem. Vürtsikiludes, mis olid tehtud külmutatud-sulatatud kalast tekkis hapu ja rääsunud maitse kiiremini kui värskest kalast tehtud proovides.

Plastikpakendis vürtsikilus moodustusid vabad aminohapped kiiremini kui klaaspakendis. Kaheksandal valmimisnädal olid plastikpakendis vürtsikilud pehmemad (madalam kõvadus), hapumad ja rääsunumad kui sama katsepunkti klaaspakendis vürtsikilud. See asjaolu on põhjustatud suuremast mikrobioloogilisest aktiivsusest, mis on tekkinud hapniku paremast kättesaadavusest.

Vürtsikilus tuvastati kolm bakteriliiki, mille arv oli üle 10^6 CFU·g^{-1}: *Brochothrix thermospacta*, *Lactobacillus sakei* ja *Aerococcus viridians*. Erinevates proovides ja katsepunktides domineerisid erinevad bakteriliigid. See on seletatav kilu mikrobioloogilise koostise heterogeensusega. Külmutatud-sulatatud toorainest valmistatud vürtsikilus ei leidunud *Aerococcus viridians*, mis viitab külmutamise potentsiaalselt negatiivsele mõjule sellele liigile. *Lactobacillus sakei* arvukus suurenes vürtsikilu valmimisaja kasvades. Saadud uuringutulemused pigem näitavad, et *Lactobacillus sakei* põhjustab vürtsikilu riknemist, mitte ei aita kaasa valmimisel.

Vürtsikilutoodete meeldivuse uuring, mis viidi läbi traditsioonilisel turul — Eestis — ja uuel turul — Taimaal — näitas, et vürtsikilu sai madalama hinnangu uuel turul, kuid seda oli ka oodata. Siiski indikeeris meeldivuse uuring, et vürtsikilutootetel on potentsiaali saada aktsepteeritud Tai tarbijate poolt, näiteks toiduretseptide koostisosana kui viia läbi vürtsikilu tootearendus (vähendada soolasisaldust ja suurendada happesust).

LIST OF PUBLICATIONS

The following publications form the basis of this dissertation and are reproduced in the appendices with permission from the publishers.

I Timberg L, Koppel K, Kuldjärv R, Paalme T. **Sensory and chemical properties of Baltic sprat (*Sprattus sprattus balticus*) and Baltic herring (*Clupea harengus membras*) in different catching seasons.** *Agronomy Research*, 9:489-494 (2011)

II Timberg L, Koppel K, Kuldjärv R, Paalme T. **Spice-cured sprats ripening and sensory properties development.** Accepted by *Journal of Aquatic Food Product Technology* 6 May, 2012

III Timberg L, Koppel K, Kuldjärv R, Chambers IV E, Soontrunnarudrungsri A, Suwonsichon S, Paalme T. **Seasoned sprat products acceptance in Estonia and in Thailand.** Accepted by *Journal of Aquatic Food Product Technology* 6 September, 2012

IV Timberg L, Jakimtšuk A, Viiard E, Koppel K, Kuldjärv R, Sarand I, Paalme T. **The effect of freezing-thawing and packaging material on the microbial dynamics of spice-cured sprats.** Submitted to *Journal of Applied Microbiology* August 2012

V Timberg L, Koppel K, Kuldjärv R, Paalme T. **Rainbow trout composition and fatty acid composition in Estonia.** *Agronomy Research*, 9:495-500 (2011)

SUMMARY OF AUTHOR'S CONTRIBUTION

I In Publication I, the author designed the study, collected and prepared samples for the experimental work, interpreted data, wrote the manuscript and is the corresponding author.

II In Publication II, the author designed the study, collected and prepared samples for the experimental work, interpreted data, wrote the text, prepared the figures, and is the corresponding author. All authors contributed to the manuscript.

III In Publication III, the author designed the study, performed the experimental work, interpreted data, wrote the text, prepared the figures, and is the corresponding author. All authors contributed to the manuscript.

IV In Publication IV, the author designed the study, prepared samples for the experimental work, interpreted data, wrote the text, prepared the figures, and is the corresponding author. All authors contributed to the manuscript.

V In Publication V, the author designed the study, collected and prepared samples for the experimental work, interpreted data, wrote the manuscript and is the corresponding author.

LIST OF PRESENTATIONS

I Timberg L, Paalme T, Kaseleht K. **Fatty acid composition of spice cured Baltic sprats, influence of diffrent types of packages and production technologies on PUFA content.** *37th WEFTA (West European Fish Technology Association) Congress*, 24-27 October, 2007, Lisbon, Portugal.

II Timberg L, Koppel K, Shpilev H, Kuldjärv R, Jakimtšuk A, Paalme T. **Sensory and chemical-biological properties of Baltic sprat (*Sprattus sprattus balticus*) and Baltic herring (*Clupea harengus membras*) in different catching seasons.** *3rd Joint Trans-Atlantic Fisheries Technology Conference*, 15-18 September, 2009, Copenhagen, Denmark.

III Timberg L, Koppel K, Jakimtšuk A, Sarand I, Paalme T. **Fishing season, raw material treatment and package influence on spice cured Baltic sprat quality properties.** *40th WEFTA (West European Fish Technology Association) Congress*, 4-7 October, 2010, Izmir, Turkey.

IV Timberg L, Koppel K, Kuldjärv R, Chambers IV E, Soontrunnarudrungsri A. **Sensory Properties and Acceptance of Spice-cured Fish Products in Thailand and Estonia.** *Pangborn Sensory Science Symposium*, 4-8 September, 2011, Toronto, Canada.

V Timberg L, Koppel K, Kuldjärv R, Paalme T. **Estonian farmed rainbow trout competitiveness in red fish market.** *41st WEFTA (West European Fish Technology Association) Congress*, 27-30 September, 2011, Gothenburg, Sweden.

VI Timberg L, Koppel K, Kuldjärv R, Paalme T. **Rainbow trout composition and fatty acid content in Estonia.** *Flavoure Confrence*, 26-27 October, 2011, Tallinn, Estonia.

VII Timberg L. **Spice-Cured Sprats Ripening, Flavor Development and Quality Properties.** *4th TAFT (Joint Trans-Atlantic Fisheries Technology) Congress*, 30 October - 2 November, 2012, Tampa, Florida, USA.

ADDITIONAL PUBLICATIONS & CONFERENCE PRESENTATIONS

A Research project on **Baltic herring and Baltic sprat development potential evaluation**. January 2008 – July 2009. Financed by Estonian Ministry of Agriculture. Reports available at: http://www.agri.ee/uuringud-3/ (in Estonian).

B Research project on **Rainbow Trout Aquaculture in Estonia**. January 2010 – June 2011. Financed by European Fisheries Fund and Estonian Ministry of Agriculture. Reports available at: http://www.pria.ee/et/otsing/vaata/support2/vesiviljeluse/ (in Estonian).

C Research project on **Underutilized Fish Raw Material Development Possibilities**. February – May 2010. Financed by European Fisheries Fund and Estonian Ministry of Agriculture. Reports available at: http://www.pria.ee/docs/resources/4811.pdf (in Estonian).

D Timberg L, Koppel K, Kuldjärv R, Paalme T. **Red fish quality and red fish consumer studies in Estonia.** *Foodbalt*, 29-30 October, 2010, Tallinn, Estonia.

E Timberg L, Koppel K, Kuldjärv R. **Salmon and rainbow trout acceptance among Estonian consumers.** *Pangborn Sensory Science Symposium*, 4-8 September, 2011, Toronto, Canada.

F Koppel K, Timberg L, Salumets A, Paalme T. **Possibility for a strawberry sensory standard.** *Journal of Sensory Studies*, 26: 71-80, (2011).

G Timberg L, Koppel K, Kuldjärv R. **Aquaculture Technology Effect of Salmon and Rainbow Trout Quality, Sensory Properties and Acceptance.** *Poster presentation at 9th Pangborn Sensory Science Symposium*, 4-8 September, 2011, Toronto, Canada.

H Koppel K, Timberg L. **Locally Manufactured Milk Chocolate Flavour Profiles: a Comparison of Estonian, Finnish, and Latvian Products.** *Poster presentation at 5th European Conference on Sensory and Consumer Research*, 9-12 September 2012, Bern, Switzerland.

I Koppel K, Timberg L. **Raspberry Jams: Flavor and Texture Variation in a Selection of European Samples.** *Poster presentation at Sensory Society Professionals Conference*, 7-12 October 2012, Jersey City, USA.

J Koppel K, Timberg L. **Preliminary Vocabulary Development and Application on Dark Chocolate.** *Poster presentation at Sensory Society Professionals Conference*, 7-12 October 2012, Jersey City, USA.

K Koppel K, Jenkins A, Vazquez-Araujo L, Timberg L, Paalme T, Carbonell-Barrachina AA, Chambers IV E. **Acceptance of Pomegranate Juice in Estonia, Thailand, Spain, and USA.** Submitted to *Food Quality and Preference.*

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ACRONYMS

AEDA	aroma extraction dilution analysis
AHC	agglomerative hierarchical clustering
ANOVA	analysis of variance
B/A	basic/acidic amino acid ratio
BLAST	basic local alignment search tool
CFU	colony forming unit
CLT	central location trial
DA	Descriptive Analysis
FAA	free amino acid
FAME	fatty acid methyl ester
FID	flame ionization detector
GC	gas chromatography
ICES	International Council for the Exploration of the Sea
LAB	lactic acid bacteria
LMWN	low molecular weight nitrogen compound
LPFP	lightly preserved fish product
MC	manual clustering
16S rDNA	small subunit ribosomal ribonucleic acid gene method
MUFA	mono-unsaturated fatty acid
n-3	unsaturated fatty acids with a double bond after the third carbon atom from the end of the carbon chain
n-6	same as n-3 with the sixth carbon instead of the third carbon
PCA	principal component analysis
PDA	photo diode array
PCDD	polychlorinated dibenzo-p-dioxin
PCDF	polychlorinated dibenzofuran
PLSR	partial least squares regression
PUFA	poly-unsaturated fatty acid
QIM	Quality Index Method
Rep-PCR	repetitive extragenic palindromic sequence polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SFA	saturated fatty acid
w/w	wet weight

CHEMICALS AND ENZYMES

ATP	adenosine-5′-triphosphate, PubChem: 5957
DNA	deoxyribonucleic acid, PubChem: 44135672
TCA	trichloroacetic acid, PubChem: 6421

THESIS

1

INTRODUCTION

The world population is rapidly growing and better utilization of all food raw materials is an ongoing challenge. The stocks of wild fish are decreasing, and thus also fishing quotas. Sprat (*Sprattus sprattus balticus*) fishing makes up 60% (47300 tons, year 2009) of the total Estonian fish catch from the Baltic Sea. Only about 1/3 of this catch is processed into added value products *e.g.*, spice-cured sprats, and the remainder 2/3 is frozen and sold as a commodity product for non-food purposes such as fish meal and animal feed (www.agri.ee/uuringud-3/, Estonian Ministry of Agriculture, 2009).

Thus, it is important to generate scientific knowledge of underutilized fish species such as Baltic sprats and further develop traditional products prepared from these species, so that they may be more widely consumed by the general public.

A globalized world creates opportunities for traditional foods that are regionally specific to branch into new markets. Traditional foods are based on local raw materials which may be unfamiliar to foreign consumers. The experience obtained during centuries of practice enables one to produce and present a traditional food with its best sensory and preservation properties. Although traditional products are very popular in local markets they may need some adjustments in order to be marketable internationally. Consumers nutritional awareness and search for new flavor experiences, in addition to demand for healthy products, generates an opportunity for unfamiliar food to be accepted.

Fish generally has a very positive image in the mind of consumers in terms of flavor and nutritional properties. It is well known that fish are an excellent source of essential amino and fatty acids. Underutilized fish species for food, such as Baltic sprat, have great potential to be embraced by consumers. Despite their very good sensory properties, the use of Baltic sprats as an everyday food has been limited because of their small size and thus time consuming preparation, which also increases the cost of commercial products.

The potential of using Baltic sprats as food was realized during Soviet period when large amounts of low added value canned products (*e.g.*, sprats in different sauces, sprat pates) in addition to traditional high added value products (*e.g.*, smoked sprats in oil, spice cured sprats) were introduced to the market. With the decrease in demand for those low added value products, significant resources of this nutritionally valuable and delicious fish have remained unexplored for purposes of food consumption. The solution may be to increase the production of high added value traditional products rather than low added value products.

Spice-cured sprats, together with smoked Baltic sprats are traditional fish products that have high commercial potential and are made from the underutilized fish species *Sprattus sprattus balticus*. Spice-cured sprats have been very distinguished products since

the 1930s when they were nominated as the best European fish product, and they were also one of the finalists in the Brussels Seafood Prix d'Elite 2012 [1].

Within the last years, the variability of spice-cured sprat products has increased significantly. Filleted products and products in plastic rather than tin and glass containers have been introduced to the market. Fish from different seasonal catches and frozen-thawed fish have been used in the curing process. All of these changes may be related to a decrease in the sensory properties and shelf life of these products.

Taken together, these factors presented a need to study the spice curing of sprats. The approach presented in this thesis contributes to both the scientific understanding of spice-cured sprat ripening process, and has practical applications in manufacturing products with high quality properties. The sensory properties of a product are critical from the point of view of consumers. Thus, this dissertation along with the publications it is based on, combines advanced biological, instrumental, and sensory analysis to gain insight into the complex process of Baltic sprat curing. Furthermore, this dissertation provides a sensory study of the potential acceptance of spice-cured sprats into new markets.

2

LITERATURE REVIEW

Baltic sprat (*Sprattus sprattus balticus*) is a herring-like, pelagic fish in the family of *Clupeidae* and is a subspecies of *Sprattus sprattus*. Baltic sprat. Its most well known relatives are herring (*Clupea*), and sardines (*Sardina*). *Sprattus sprattus* is found in the north-east Atlantic Ocean and most of the Baltic Sea, while the area of Baltic sprats is limited to the Gulf of Riga, Gulf of Finland, Northern Baltic Proper, and Eastern Gotland Basin. The body of *Sprattus* is covered with silver-gray scales and the flesh is white-grey. The average length of Baltic sprats is 12.0 cm, average weight 12.0 g, and age when caught is 2-3 years [2]. The lipid content of Baltic sprat may be as low as 10.0% and can reach levels of over 20.0% [2–4]. Baltic sprat feeds on planktonic crustaceans, and spawns in June and July. The schools brake up after spawning. Thus, Baltic sprat can be caught only in autumn, winter and spring. Baltic sprats caught by Estonian vessels are mostly habitants of the Gulf of Riga, Gulf of Finland, and Eastern Gotland Basin.

The ecological state of the Baltic Sea raises a concern that fish may contain the dioxins polychlorinated polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) [2, 4]. However, it has been demonstrated by [2] that the concentration of PCDDs and PCDFs in sprats increases with the age and exceeds the EU maximum limit value in fish older than 5 years. Because most of the sprats caught are only 2 to 3 years old, their should be no concern with regards to dioxin concentration for Baltic sprat.

2.1 BALTIC SPRATS AS A STABLE FOOD SOURCE

Baltic sprat, together with baltic herring are traditionally consumed in the Baltic Sea coast area. However, both of these fish species are caught in large numbers, and thus it is impossible to consume all of them as fresh fish. Traditionally, salting and smoking have been used to preserve these fish. Canning and freezing of fish became predominant methods for fish preservation, and now newer technologies are being adopted. Salted Baltic sprats have usually been packed into barrels or tins, because these are the most affordable packaging methods which also provides a long shelf-life.

Three of the most popular Baltic sprat products in Eastern Europe and other European countries are spice-cured sprats, smoked sprats in oil, and sprats in tomato sauce. Smoked sprats in oil and sprats in tomato sauce are canned, heat treated at 121℃, and stored at room temperature. In contrast, spice-cured sprats are preserved without heat treatment and contain active enzymes and microbes throughout shelf-life of the product. The tech-

nological process with no heat treatment makes controlling the properties of spice-cured sprats a more challenging task.

Traditionally, spice-cured sprats were made only from autumn caught sprats. It was known by empirical observations that sprats from other seasons do not provide the same sensory properties. Krosing and Kask [5] demonstrate that the digestive enzymes of sprats have a seasonal variation, and their activity is highest in autumn fish.

The classical product of spice-cured sprats is "Sprats of Tallinn". According to the manufacturing technology, whole sprats were layered into metal tins, with spice-mix added in between layers of fish. The tins were filled with salt brine (Figure 1) and sealed hermetically. The sprats were ripened for two weeks at 2-4℃. After this time period sprats were considered ripened and they were marketed for up to 3 months. To both reduce the production costs and provide the product all year round, current spice-cured sprat production are different. In addition to the use of more appealing packaging materials, filleting is applied, which can have an effect on flavor properties and the overall product quality.

Figure 1 – Production scheme of spice-cured sprats "Sprats of Tallinn".

2.2 CURED FISH PRODUCTS ARE CONSUMED GLOBALLY

Fish curing has been used for centuries. In this process whole, partially gutted or gutted fish is salted and ripened until the fish obtains a pleasant flavor and texture [6]. Curing

can be carried out with only salt, or salt in combination with sugar and/or spices. Although the role of curing in fish preservation has decreased, cured fish products are still being widely produced because this process enables the fish to develop desirable sensory properties. Well known cured products are maatjes herring from the Netherlands [7, 8], salted herring from Northern Europe [6, 9], and anchovies and sardines from Southern Europe [10]. Curing technologies depends on the fish species used and local traditions. Most cured fish products today are refrigerated to increase the shelf-life.

Maatjes herring, a lightly-salted ready-to-eat fish product is made from herrings just before spawning between May and July and is characterized by a distinct level of subcutaneous fat of 16-20%. Herring is gibbed (removal of the gills and part of the intestine) with only the pancreas left intact to provide the enzymes needed for curing. They are salted in a barrel for one to four days, depending on their size and result in a NaCl concentration of 2.0% wet weight (w/w) in the final product. After ripening, the product is filleted and consumed or frozen for storage [8].

Salted Northern Europe herring is also made only from fatty herring (less than 15% fat) and for spring-spawning herring the catch period is typically from October to January. Herring is beheaded, salted, sugar-salted or spice-salted and stored for a few days at 10°C until the brine is formed. Saturated brine is added to exclude all air and the herring is pressed down so that it will be completely covered by the brine. The herrings are then left to cure for up to 1.5 years. Curing time depends on the storage temperature (-2 to 6°C), the salt content (7-18% w/w), and the final product [6, 9, 11].

Anchovies (*Engraulis encrasicholus*) and sardines (*Sardina pilchardus*) from Southern Europe and Southern America are also used for curing just before spawning, when they have their highest fat content (>4.0% and >10.0%, respectively). The fish is immersed in saturated brine, then beheaded and partially gutted (nobbed) and packed in barrels with layers of salt and fish (0.2:1; w/w). As with herring, they are pressed down and covered with saturated brine. The fish are left to cure at room temperature (18-22°C) for up to 1 year, and when mature they are filleted and packed in oil or salt or processed further. The salt content of these fish is about 19% [12, 13].

2.3 SALTING AND RIPENING

The salting and ripening processes of these fish can be divided into two separate stages. Salting is an osmotic dehydration process which is divided into dynamic and equilibrium phases. In the dynamic phase, the mass transfer of water and salt occurs until equilibrium is reached. Equilibrium is the end point of osmosis when the net rate of mass transport is zero. Salt has an impact on muscle structure by inducing swelling and aggregation [14, 15].

The structure and biological activity of proteins are influenced in two different ways by salts, a nonspecific (electrostatic) effect, and an ion specific (lyotropic) effect. At low salt concentrations (<1.2%), the effect of salts are considered to be purely electrostatic, where ions bind non-specifically to charged groups on the protein surface and thereby affect the electrostatic interactions within the protein and between the protein and the solvent. In contrast, when the salt content exceeds 1.2%, the electrostatic effect is assumed to be saturated and the protein is influenced by an ion specific effect known as the lyotropic effect

[16, 17]. The lyotropic effect is mainly caused by Na^+ and Cl^- and can be described by its ability to act as a solubilizing (salting-in) or a precipitating (salting out) agent on proteins [18]. Salt in high concentrations influences the organization of water molecules surrounding the protein and thereby the solubility of hydrophobic groups both inside the protein and on the surface of the protein decreases which favors the native state of the protein [17]. The salt concentration exceeds the concentration of the electrostatic effect within a short time in cured fish products and the main effect expected is the lyotropic effect [6]. In salting processes, proteins generally first show an increase in solubility (salting-in) and, upon further addition of salt, the solubility decreases (salting-out). Sodium chloride at concentrations of from 0.8-2.8% induces a salting-in effect of myofibrillar proteins [15].

Salt absorption is affected by brine concentration and temperature, the time within the brine solution, the thickness and geometry of fish, the texture and fat content of fish, and the species and quality of fish [19]. The myosin proteins in fish muscle denature when the salt content is higher than 8-10%. Other muscle myofibrillar proteins are partially denature at salt content <16% and totally denature when it is higher than 20% [20]. Collagen, the main protein in connective tissue, is partly soluble in neutral salt solutions and swells at concentrations <2.8% [21, 22]. Moreover, studies on cattle collagen show that the maximum solubility [23] is obtained at similar levels (2.2%) as for myofibrillar proteins [24].

Soft-textured fish *e.g.*, anchovies and sprat, tend to absorb salt faster than firm-textured fish [25]. Thus, salting-in is a relatively fast process, especially in the case of smaller soft-textured fish and it reaches equilibrium within the first month of ripening. Only a slight increase in salt concentration of the fish can be observed in the subsequent curing stage [12]. Fish with a high fat content absorb salt slower than low-fat fish [25]. Frozen-thawed or low-quality fish muscle cells might be broken and this may increase the rate of salt absorption [26].

2.3.1 *Role of enzymes during ripening*

The term ripening or curing, describes the process of desirable proteolytic and lipolytic changes. Fish muscle and viscera both contain a number of proteases. In the process of filleting fish, salt curing endogenous enzymes of fish muscle are mainly responsible for the weakening of the myofibrillar structure. It has been shown that these post mortem proteolytic changes are mainly caused by lysosomal cathepsins — calpains and proteasomes [27]. Salting influences the enzymatic activity responsible for degradation of structural components in fish muscle [27]. It has been demonstrated that in salt curing of cod (*Gadus morhua*) the enzymatic activity chymotrypsine, trypsine, collagenase and elastase) was stimulated with increasing salt concentration (up to 1 M NaCl), but declined when the salt curing proceeded and the salt content rose above 1 M [28].

Several studies have shown that proteases and peptidases secreted by the mucosa of the small intestine, pancreas and especially appendices pyloricae play an important role in the ripening of fish [6, 9, 12]. Telost fish species, *e.g.*, herring, sardine, anchovies, and sprat, do not have a discrete pancreas which secrete digestive enzymes, but have pancreatic cells diffused around the pyloric cacea and intestine containing the digestive

enzymes [29, 30]. Enzymes stored in organs and tissues responsible for synthesis and release of digestive enzymes into the muscle of fish and into the brine takes place within a few hours after the death of the fish.

Fish enzymes in general resemble the corresponding proteases from mammals, except they are adapted to the lower habitat temperatures [31]. The activity of fish proteases at low temperatures is also important in ripening, which is usually carried out at temperatures ranging from -2 to 6℃. A number of endopeptidases have been isolated and studied from many different fish species, but only a few studies on exopeptidases from fish have been carried out [6]. In addition to the adaptation to be active at lower temperatures, some enzymes are also active at high NaCl solutions and their activities vary between different seasons. Vo *et al.* [32] showed that aminopetidases from sardine intestine remained stable and active in a 20% NaCl solution. This finding suggests that amino acids can be released from fish proteins at high salt concentrations and can therefore influence the ripening process of fish. Comparison of the proteolytic activity in brine and muscle from salted gutted and ungutted herring shows a higher proteolytic activity in both brine and muscle tissue from ungutted herring compared to gutted herring [6]. Krossing and Kask [5] demonstrated that in the spring when the proteolytic activity in sprat gut is lowest, proteins do not decompose and the sprat failed to ripen. This indicates that a diffusion of digestive proteases into the muscle takes place and the presence of viscera and intestines causes an increase in the amount of low-molecular-weight compounds. This effect contributes to their characteristic flavor.

Previous research has shown that enzymatic ripening by intestine and muscle proteases is one of the key elements of fish curing, and because many different proteases are active simultaneously, it is very likely that they all play a vital role in the curing process and in the development of characteristic flavors. The activity of proteases depends upon the feeding and maturation cycle of fish, and needs to be taken into consideration when studying fish curing.

The proteolytic activity of fish varies considerably during the year. Changes in feed intake may cause higher proteolytic activity during certain periods [6]. High fat content is a critical quality indicator for all cured fish products because it correlates with desirable sensory properties. It has been shown that chemical and enzymatic oxidation of unsaturated fatty acids and further interactions with proteins, peptides, and free amino acids creates the typical ripened products flavor softening of texture [11, 24, 33]. High fat content is considered as an indirect indicator of digestive enzymes with high activity [6]. Krosing and Kask [5] and Krosing and Veldre [3] also report that spring sprats have low fat content (<10%) and almost no proteolytic activity, while winter sprats have a higher fat content (20%) and higher proteolytic activity. This supports the hypothesis of the importance of fat content as an indicator of digestive enzymes with high activity.

2.3.2 *Role of peptides and free amino acids during ripening*

Enzymes from fish viscera and muscle cut proteins into peptides and peptides into amino acids (Figure 2). Thus peptide and free amino acid content of cured fish and brine have been used to characterize the extent of ripening [34]. The methods mainly used for de-

termining low molecular weight nitrogen compounds (LMWNs) in ripened fish products are: trichloroacetic acid (TCA) soluble nitrogen compounds (TCA index); free amino acid (FAA) content; and soluble protein pattern using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [6, 34–37].

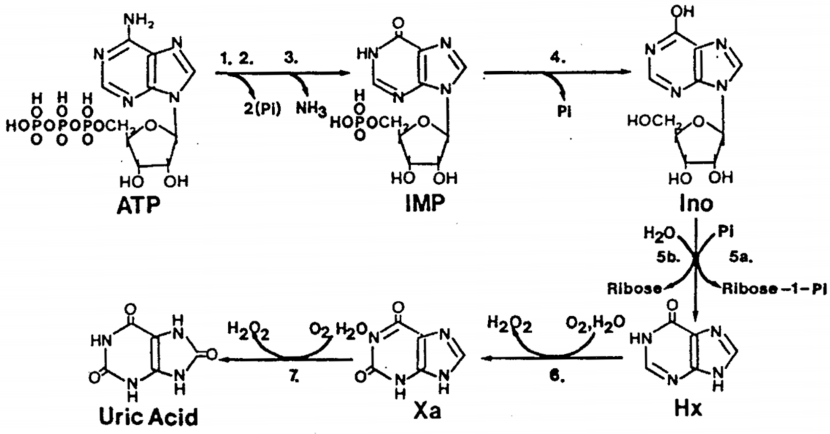

Figure 2 – *Post mortem* ATP degradation in fish muscle. Enzymes include: 1. ATPase; 2. myokinase; 3. AMP deaminase; 4. IMP phosphohydrolase; 5a. nucleoside phosphorylase; 5b. inosine nucleosidase; 6, 7. xanthine oxidase [38].

2.3.2.1 *TCA index*

The amount of TCA-soluble nitrogen is often given as a percentage of the total nitrogen content. The TCA index of fish ripening studies shows that there is a large increase in LMWNs during ripening [6, 34]. However, it is not certain that all compounds represented in the TCA index contribute to the taste of ripening fish, thus these methods cannot be used directly as an expression of ripening profile [6]. The TCA index increases during ripening. The value depends on the fish species, enzymatic activity, and also may be influenced by fat content. Optimal TCA values around 30% have been found for sardine [12], and herring [11] ripening. Higher values were obtained in fresh fish ripening, compared with frozen-thawed fish, which indicates faster proteolytic breakdown. The TCA index can only be used effectively in ripening studies if there are previous results available on the fish species under study.

2.3.2.2 *Free amino acids (FAAs)*

The analysis of FAAs provides a holistic picture of the amount of end products found after ripening. The content of FAAs is widely used because Kiesvaara [34], and several other researchers [12, 39, 40], have found that an observed decrease in the ratio between basic and acidic amino acids (B/A) correlates well with curing time and sensory properties. In

many studies fish was considered ripened when the B/A was around 1.0 [12, 34, 39, 40]. The use of FAA analysis may be a valuable tool to compare different studies on fish ripening.

2.3.2.3 *Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)*

Using SDS-PAGE enables one to visualize protein bands and their degradation during the ripening of fish. SDS-PAGE also allows one to compare the profiles of soluble proteins in cured fish prepared with different gutting methods [6, 36, 37]. The SDS-PAGE profile of muscle tissue shows much weaker proteolysis compared to the proteolysis that takes place in brine solution [6]. Nielsen [6] also demonstrated that low-molecular weight proteins have a stronger intensity in the brine from ungutted herring, and this indicates a stronger activity of proteolytic enzymes. Andersen *et al.* [36] and Christensen *et al.* [37] show, using SDS-PAGE, that in herring ripening myosin and actin almost completely degrade after 371 days. However, it was not possible to detect quantifiable differences between batches. Thus, this method is difficult to utilize to compare different studies.

2.3.3 *Role of free amino acids and nucleosides during ripening*

Peptides and amino acids are formulators of flavor, and they also influence texture properties. Their role in fish ripening is thus important. inosine 5′-monophosphate (IMP) is a flavor enhancer, which contributes to umami taste [41], whereas hypoxanthine together with some free amino acids, anserine, carnosine, and other dipeptides may contribute to a bitter taste in meat [42]. Amino acids that effect fish taste most strongly are glycine (Gly), alanine (Ala), glutamic acid (Glu), histidine (His) and valine (Val) [43]. Glycine and alanine are known to have a sweet taste, while glutamic acid has an umami taste, and histidine and valine have a bitter taste. Kirimura *et al.* [44] tested 60 different peptides and found that their taste intensities were weak when compared with amino acids. Peptides with acidic residues have a sour taste, while those with hydrophobic residues have a bitter taste. Interestingly, a balanced composition has little or no taste [45].

2.4 MICROBIOLOGY OF CURED FISH

Fish contains small amounts of carbohydrates (0.2-1.5% depending on the species) and is rich in low molecular weight nitrogenous molecules (*e.g.*, FAAs, nucleotides) that are rapidly metabolized by bacteria. This makes fish an excellent matrix for microbiota development. Cured fish can still be classified as lightly preserved fish product (LPFP) if they undergo many processing stages, however, extra processing steps may increase the risk of microbial contamination. Raw material freezing and thawing, different package materials and atmospheres, and the fact that cured fish is usually eaten without further heating indicate the need for research into microbiological development in cured fish. Fish ripening processing treatments inhibit the growth of pathogenic bacteria but do not completely inhibit the growth of microorganisms. The addition of NaCl at a concentration of about 5.5-6.5% w/w, decreases the water activity (a_w). When a_w is decreased down to 0.96, Gram-negative bacteria are inhibited (*e.g.*, *Pseudomonas* spp.), however, this does not

prevent the growth of other more resistant organisms, *i. e.*, lactic acid bacteria (LAB) [46]. Many LAB strains isolated from LPFPs are known to grow in conditions up to 8-10% w/w of salt [47–49]. At the end of LPFP shelf-life Gram-positive bacteria, particularly LAB, become predominant, sometimes associated with *Enterobacteria* and *Brochothrix thermospacta* [8, 46, 50, 51]. Leroi [46] stated that even if LAB species dominate in LPFPs, their role is not very clear, because several authors have found no correlation between total LAB counts and sensory spoilage [8, 50, 52, 53]. Many studies have been conducted on the effect of individual species on LPFP sensory properties and spoilage, and it has been demonstrated that results are very species specific [54–60]. Moreover, interactions between different bacterial species and groups change their collective metabolism and thus it is impossible to predict the quality of LPFP using only one microbiological or biochemical parameter.

A number of microbiology studies have been conducted on cured fish products, including salted herring [8, 61–63] and ripened anchovies [64]. Lyhs *et al.* [8] studied maatjes herring (salt content 1-2%) stored under both air and under a modified atmosphere (at 4 and 10°C) and found that the total viable bacterial counts did not reach 10^6 CFU·g^{-1} for the day of sensory rejection. Thus it appears that microbial processes have a minor significance in the sensory assessment of maatjes herring, which is the most lightly salted cured herring product.

Microbiological studies on salted anchovy have mainly been conducted on histamine forming bacteria [64–66]. Triqui and Reineccius [33] found that microbial metabolism did not contribute to sensory volatiles when using aroma extraction dilution analysis (AEDA).

Thus, the development of cured fish microbiota is influenced by raw materials and product characteristics and must be described in the interest of shelf life, sensory properties, and population dynamics of the product.

2.5 SENSORY EVALUATION OF FISH AND CURED FISH

Sensory properties of different fish species vary greatly, and there are a number of different sensory analysis methods for evaluating fish and fish products. For fish, the most widely applied techniques are Quality Index Method (QIM) and Descriptive Analysis (DA).

2.5.1 *Quality Index Method (QIM)*

QIM is used to evaluate the freshness of fish by visual inspection by trained experts. QIM evaluates certain attributes of raw fish (skin, eyes, gills, meat, and blood occurrence) using a scoring system (0 to 3 points). The scores of all attributes are summed, which result in a quality index. The quality index increases linearly with fish storage time in ice. The description of the evaluation of each attribute is fixed in a guideline, which is dependent on species (`http://www.qim-eurofish.com`). However, QIM evaluation of Baltic sprat is not available.

Lyhs and Schelvis-Smit [67] developed a QIM scheme for maatjes herring. The following attributes were assessed: Appearance of the skin side and bone side, color of the blood, odor (rancidity and other), taste (rancidity and other), aftertaste and texture. For each attribute, scores from 0 to 3 were given. Freshly produced maatjes herring was de-

scribed by panelists as having a firm texture with a good bite, a fresh marine odor and flavor, a light salty odor and taste, and no signs of rancidity.

2.5.2 *Descriptive Analysis (DA)*

DA assessment can be accomplished both for fresh fish and processed fish, providing that a sensory profile has been established [68]. DA profile describes the intensities of different attributes which have been previously decided to be important quality parameters in each case. The DA method is time consuming and expensive, and is therefore mainly used by researchers and rarely for industrial product development.

DA method has been widely used for cured fish products, although there is no uniform DA profile, because different fish species for raw material always add some specific attributes. Filsinger's [69] scoring method has been used for cured anchovies [10, 70] and for cured sardine [12]. Filsinger's method involves evaluation of flavor, odor, flesh colour, flesh consistency, and adherence of flesh to the backbone. The mean score of these five subscales gives a degree of ripening, on a scale ranging from 0 (raw), through to 2 (initial ripening, 4 (pre-ripened), and 6 (ripened), up to 8 (spoiled).

Olsen and Skåra [40] used another scale for cured herring taste and texture attributes: ripened taste, malty, salty, sweet, spicy, aftertaste and smoothness, softness, tenderness, and intensity scores were from 1 (none) to 7 (maximum). The most characteristic sensory properties were ripened and malty flavor and smoothness.

Schubring and Oehlenschläger [71] used a DA scheme which was developed for an enzymatically ripened salted herring product, where six attributes were chosen for the assessment of odor, flavor and taste (ripeness, maltiness, rawness, sweetness, rancidity and aftertaste); and seven attributes were chosen for texture (succulence, fatty mouthfeel, softness, hardness, tenderness, dryness and toughness). For each single attribute a scale consisting of 100 points was used; 0 was defined as the minimal mark of the attribute, 100 as the maximal mark of the attribute. The study showed that a pleasant and typical ripening flavor was only detected in herring in pre-spawning state, not with herring in a spawning state.

Gudmundsdóttir and Stefánsson [72] used a DA method for spice-salted herring where eight attributes for flavor were used (ripened, raw, malty/creamy, stock fish, salty, sweet, spicy, and aftertaste) and three attributes for texture were used (softness, watery, and toughness). Each attribute was evaluated on an intensity scale anchored at both ends (0 = none to 100 = strong). The study showed that salt ripened fillets did not develop the characteristic ripened flavor whereas samples made from nobbed herring had a high intensity of ripened flavor.

A literature overview of DA for cured fish shows that many different versions of the profiles and scoring techniques have been used. Cured anchovies and sardine have mostly been studied by Filsinger's method, while DA methods and one QIM method have been used for research on cured herring. Filsinger's method provides detailed descriptions for each score point for each attribute, however, cured herring scores usually have only descriptions of the minimum and maximum value. The variation in attributes and scoring technique vary with the nature of the research being undertake, but also due to the ex-

perience and background of the sensory panel used. However, common attributes for all cured herring profiles are: ripened, raw, malty, sweet, salty, rancid, aftertaste, softness, toughness, and dryness. Finding a suitable reference material for many of those attributes can be a challenging task, and this also depends on the local food supply options. A total unification of cured fish DA scoring techniques is impossible, however, it is strongly suggested that at least within one species of cured fish one set of attributes should be used. This would allow one to consistently compare different studies and allow one to combine data from different sources.

3

AIMS OF THIS DISSERTATION

The aim of this dissertation is to study the ripening process of spice-cured sprats and identify key quality attributes and their relationships to raw material pretreatment and package materials. The specific aims are:

I To determine the seasonal composition of Baltic sprats (*Sprattus sprattus balticus*) over a number of years and evaluate how this composition influences sensory properties.

II To investigate and identify changes in the chemical composition and sensory properties during spice-curing of Baltic sprats.

III To evaluate the influence of microbial contamination within the fish on the ripening and spoilage of spice-cured sprats.

IV To evaluate the effect of fish freezing-thawing on the chemical, textural, microbiological, and sensory properties of spice-cured sprats.

V To evaluate the effect of package material (glass or plastic), on the chemical, textural, microbiological, and sensory properties of spice-cured sprats.

VI To examine the acceptance and crucial quality indicators of spice-cured sprat products for both local and foreign markets.

4

MATERIALS AND METHODS

More detailed descriptions of the materials and methods applied are available in the publications. The following sections are provided to make this material more accessible.

4.1 MATERIALS

Baltic sprat (*Sprattus sprattus balticus*) samples were collected from fish caught from February 2008 until April 2009 (Publication I). Twenty-six samples of baltic sprat were caught from the Baltic Sea. Sprat from the Gulf of Finland (International Council for the Exploration of the Sea (ICES) rectangle 32), form the Gulf of Riga (ICES rectangle 28) and from the costal line of Hiiumaa and Saaremaa (ICES rectangle 29) were caught by professional fishermen (Figure 3). Samples were harvested together with fish biologists from the Estonian Marine Institute, Tartu University (Figure 3). Samples of fish were immediately frozen after landing and stored at -18℃. The frozen fish samples were thawed at 2-4℃ for 48 hours prior to analysis. Thawed fish were de-headed, gutted and washed.

To study the ripening process, baltic sprat (*Sprattus sprattus balticus*) were caught in December 2009 and in November 2010 (Publication II and Publication III). Half of the fish (25 kg) was spice-cured the day after being caught, and half of the fish (25 kg) was frozen. A spice mixture, containing several different spices such as vanilla, cinnamon, cardamom, coriander, ginger, nutmeg, nutmeg flower, allspice, clove, black pepper, and bay leaves, was obtained from a local spice manufacturer (Paulig, Saue, Estonia).

Three spice-cured sprat products A, B, and C (Publication IV) were obtained from the Estonian fish production facilities. Product A represented the traditional preparation of spice-cured sprats, where whole sprats together with brine and seasoning are packaged in tin cans. Product B consisted of lightly spiced vacuum-packaged seasoned sprat fillets, and product C consisted of seasoned and marinated sprat fillets in oil, which were packaged in glass jars.

All necessary reagents for chemical analyses were purchased from Sigma-Aldrich, Germany. Media for microbiological analysis were obtained from LabM Ltd., UK. For DNA extraction of the isolates FTA MiniCards (GE Healthcare Ltd., UK) were used.

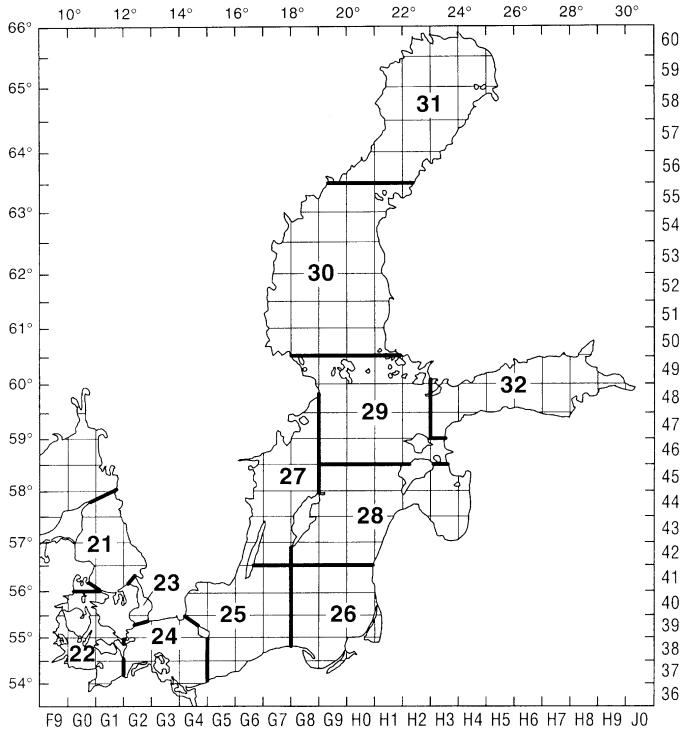

Figure 3 – ICES rectangles of baltic sprat samples catching places (`http://www.ices.dk`).

4.2 METHODS

4.2.1 *The ripening process*

A laboratory curing process was designed on the basis of an historical recipe of "Sprats of Tallinn" to study the effect of individual technological parameters. Whole sprats (including heads and guts) were layered into 185 ml glass and plastic jars together with the spice-mix (1.7 g / 100 g of fish), covered with brine (saturated NaCl solution) to remove air, and sealed with metal or plastic caps (Publication II and Publication IV). The ratio of fish and brine in the jars was 70:30. Preserved sprats were cured at 2-4℃ and analyzed from two to ten weeks. Frozen sprats were kept at -18℃ for three months, after which they were thawed at 2-4℃ during 24 hours, and prepared and packed into glass and plastic jars using the same method as with the fresh fish. These procedures correspond to industrial practices for the production of spice-cured sprats. The samples were stored at 2-4℃ before evaluation.

4.2.2 *Sampling*

Water, lipid, protein, ash, pH, free amino acids, and fatty acids of the samples were measured in triplicate and the results were averaged (Publication I, Publication II and Publication III). All samples (*ca* 300 g of fish from three different jars) were filleted, drained of excess fluids, and minced.

Samples for sensory analysis were encoded with three-digit numbers, and analyzed in two (Publication I) or three (Publication II, Publication III and Publication IV) repetitions. In Publication I the fish were frozen immediately after landing, and prior to analysis the frozen fish samples were thawed at 4℃ for 48 hours. Thawed fish were de-headed, gutted and washed. Samples for sensory analysis were packed into aluminum foil, steamed for 10 minutes at 65℃, and served immediately.

For the evaluation of spice-cured sprat samples, each panelist was served two whole fish (Publication II, Publication III and Publication IV). The serving of the samples was randomized. The samples were prepared 30 minutes prior and references 30 minutes to two hours prior to testing. The samples were stored at 2-6℃ before evaluation. Spice-cured sprat samples for consumers were all filleted, coded with three-digit numbers and served in a randomized order. The samples were stored at 2-6℃ prior to evaluation.

Samples for microbiological analysis in Publication IV were made with 175 grams of whole fish, because this was the amount of fish in one jar. A volume of 70 ml of 0.85% NaCl solution was added into a Stomacher bag after adding the equivalent of one jar of whole fish (175 g fresh or thawed). The mixture was homogenized in a Stomacher 400 circulator (Seward Ltd., UK). Decimal dilutions were made from the fish mixture and plated on MRS [73] , MRS + 7% NaCl, and plate count agar. The plates were incubated in anaerobic conditions at 22℃ for 3 days.

4.2.3 *Biological measurements*

The total weight and length of all fish were measured to the nearest of 0.1 g and 1 mm, respectively. The otoliths of fish were taken for age determination. The fish age was determined as the number of hyaline rings with individuals being moved to the next age group on the 1st of January.

4.2.4 *Chemical and physical methods*

The water content of the minced fillets was measured using a halogen analyzer (HR 83, Mettler Toledo, Switzerland), and protein content by the Kjeldahl method (Velp Scientifica UDK 142, Italy). For pH measurements the minced fish was diluted with distilled water (1:10) and homogenized, and measured with a 744 pH Meter (Metrohm, Switzerland). Samples for lipid analysis were freeze-dried and measured by the Soxhlet method (Velp Scientifica SER 148 Solvent Extractor, Italy).

For amino acid analysis the homogenized samples were frozen at -40℃ and freeze-dried (Heto PowerDry PL3000, HSC500, Denmark). Chromatographic analysis was carried out using an ACQUITY UPLC® system (Waters, USA), equipped with a C18 column

(Waters, USA) and a photo diode array (PDA) detector ACQUITY PDA 2996. Amino acid standards used for external calibration were obtained from Serva (Germany). The fatty acid profile of sprat samples fillets was determined after derivation of lipids into fatty acid methyl esters (FAMEs) according to the standard EVS-EN ISO 5509:2000. Bligh & Dyer [74] method was used for lipid extraction. Chromatographical analysis was carried out using an Agilent 7890A GC system equipped with the flame ionization detector (FID), and Agilent J&W GC Column HP-88. External standard (Supelco 37 Component FAME mix, Sigma-Aldrich, Germany) were used to quantify chromatographic peaks of the sample.

Texture analysis was preformed by small deformation rheological analysis according to [75] (Publication II). Minced fish paste was prepared by mixing 90 g minced fish with 10ml distilled water and homogenized at 11000 rpm for 2 minutes (Polytron PT 2100, Kinematica, Switzerland). The sample was measured with a rheometer (Physica MCR301, Anton Paar, Austria).

4.2.5 *Microbial analysis*

The plating of sprat samples was analyzed in triplicate on MRS, plate count agar and MRS + 7% NaCl media. The colonies (10-20 from each sample point) were picked from plates and grown in liquid media. FTA MiniCards (GE Healthcare Ltd., UK) were used for DNA extraction of the isolates according to the manufacturer's instructions. Repetitive extragenic palindromic sequence polymerase chain reaction (Rep-PCR) with primer (GTG)5 was performed essentially as described by De Vuyst *et al.* [76]. Representatives of each fingerprint group were detected by Rep-PCR were subjected to 16S rDNA gene sequencing. The partial 16S rDNA sequences were compared with GenBank database using basic local alignment search tool (BLAST) (National Center for Biotechnology Information, USA) for finding the closest match. More detailed information about sample preparation and analysis is described in Publication IV.

4.2.6 *Descriptive sensory methods*

Descriptive analysis by 6 trained panelists was used to evaluate the sensory properties of sprat and spice-cured sprat samples in Publication I, Publication II, Publication III and Publication IV. Two descriptive analysis vocabularies were developed and used, one for sprat (Publication I) and the other for spice-cured sprat products (Publication II, Publication III, Publication IV) evaluation. Attributes for sprat descriptive analysis were: appearance (skin color, meat color, gapping, broken skin, shape), flavor (off-flavor, characteristic flavor, off-aroma, characteristic aroma, sweetness), and texture (hardness, bone separation, cohesiveness, moistness, greasiness). Attributes for spice-cured sprat products descriptive analysis were: appearance (shininess, meat color), flavour (overall spiciness, allspice, nutmeg, nutmeg flower, cinnamon, bay leaves, black pepper, vanilla, clove, ginger, coriander, cardamom, fish, sweet, sour, salty, rancid), texture (hardness, moistness, and greasiness). The samples were evaluated on a 9-point (Publication I) or 15-point (Publication II, Publication III, Publication IV) numerical scale, anchored at both ends. Unsalted crackers and purified water were available for palate cleansing.

4.2.7 *Consumer acceptance study*

The central location trials (CLTs) [77, 78] were carried out in the Competence Center of Food and Fermentation Technologies (CCFFT), Tallinn, Estonia, and in the Kasetsart University, Sensory and Consumer Research Center, Bangkok, Thailand (Publication IV). The consumers were asked to fill in a questionnaire with questions about overall liking, appearance, aroma, texture, flavor, fish flavor, salty taste, and aftertaste liking; "Just About Right" questions on intensities; check-all-that-apply (CATA) statements on attitudes, and demographics.

4.2.8 *Data analysis*

One-way analysis of variance (ANOVA) was used to detect statistically significant ($p < 0.05$) differences between samples (Publication I, Publication II, Publication III and Publication IV) and differences between consumer clusters (Publication III).

Mapping of samples as biplots according to average attribute scores was performed using principal component analysis (PCA). In PCA biplots the sample scores and variable loadings were visualized on the same map. Partial least squares regression (PLSR) was used to map connections between instrumental composition measurements and sensory analysis data.

Correlations between attributes were found using Pearson correlation coefficient ($p = 0.05$). A similar approach has been used by Koppel and Chambers [79], Torrieri *et al.* [80] and others. Consumer clusters were determined with agglomerative hierarchical clustering (AHC) and manual clustering (MC) methods in Publication III.

XL Stat (AddInSoft, New York, NY, US), and Unscrambler (Camo Software, Norway) was used in the data analysis.

5

RESULTS

This dissertation is being defended on the basis of five publications, the results of which are summarized below.

5.1 BALTIC SPRAT COMPOSITION AND ITS SENSORY CHARACTERISTICS

It has been shown that fat content of fatty fish is influenced by different factors, such as age, maturation, and season. Fat is an important parameter in creating sensory perception such as full and succulent mouthfeel. This was demonstrated also in a comparative study of Estonian and Finnish farmed rainbow trouts (Publication V). Below the results of the study on composition and sensory properties of the Baltic sprat are summarized (Publication I). For this work sprat samples were caught at different seasons from selected Estonian fishing grounds that are the most commonly used for producing sprat products. The average age of the sprat caught was 2.5 years. The age was below two years (1.2-1.9 years) in the autumn catch and higher than two years for all other catches (Figure 4). Thus, the Baltic sprat caught from Estonian waters is very young, and consumption of these fish and products made from them carries a low risk of dioxin contamination [2, 81]. The body mass index of the sprat is highest during autumn (average 0.84 $g \cdot cm^{-1}$), and it decreases during winter (average 0.80 $g \cdot cm^{-1}$) because of a lack of food, and continues decreasing towards the spring (average 0.70 $g \cdot cm^{-1}$) (Figure 4).

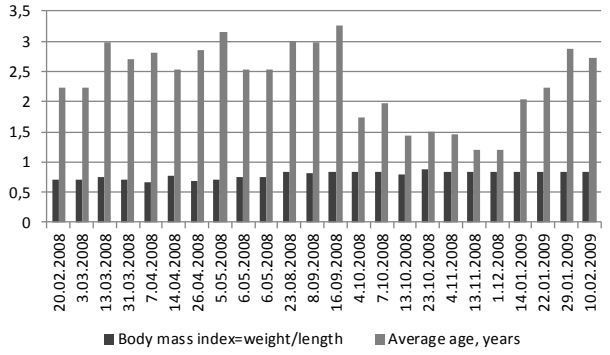

Figure 4 – Average age (years), and average body mass index ($g \cdot cm^{-1}$) of Baltic sprat.

The composition of sprat varies in fat and water content, while protein content (14.8% ± 0.59) remains constant (Publication I, Figure I and Publication II, Table 1). There is an inverse relationship between the water content and the fat content (Publication I). Samples of fish from the autumn catch (September – December) contained 50-60% water, while the spring catch (March – May) contained 61-67% water. In these same two catches, the fat content was found to be 16-22% and 10-16%, respectively.

Table 1 – Fatty acid composition ($g \cdot g_{lipid}^{-1}$) in Baltic sprat and in rainbow trout farmed in Estonia and in Finland. Values are mean ± standard error.

Fatty acid	Baltic sprat	Rainbow trout farmed in Estonia	Rainbow trout farmed in Finland	Rainbow trout average
14:0	5.0 ± 0.3	4.6 ± 0.5	4.5 ± 0.5	4.5 ± 0.5
16:0	22.1 ± 0.5	16.4 ± 1.1	14.0 ± 1.0	15.2 ± 1.0
18:0	2.2 ± 0.0	2.9 ± 0.3	3.2 ± 0.6	3.1 ± 0.4
Σ SFA	29.3 ± 0.7	23.9 ± 0.6	21.7 ± 0.7	22.8 ± 0.6
14:1	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
16:1	5.9 ± 0.2	5.2 ± 0.3	5.4 ± 0.5	5.3 ± 0.4
18:1	24.5 ± 0.8	26.7 ± 1.1	29.7 ± 1.2	28.2 ± 1.1
20:1	0.5 ± 0.0	3.0 ± 0.5	4.3 ± 0.7	3.7 ± 0.5
22:1	0.7 ± 0.0	0.7 ± 0.1	0.6 ± 0.0	0.7 ± 0.1
Σ MUFA	31.8 ± 0.8	35.8 ± 0.8	40.0 ± 1.5	37.9 ± 1.1
18:2n6	3.1 ± 0.1	9.0 ± 1.1	11.1 ± 0.8	10.0 ± 0.9
18:3n6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:3n3	3.1 ± 0.1	3,4 ± 0.5	3.7 ± 0.5	3.5 ± 0.5
20:2n6	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
20:3n3	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
20:5n3	9.1 ± 0.1	5.8 ± 0.7	5.0 ± 0.2	5.4 ± 0.6
22:2n6	0.6 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	0.4 ± 0.0
22:6n3	22.1 ± 0.5	20.1 ± 1.3	16.4 ± 0.9	18.2 ± 1.0
Σ PUFA	38.9 ± 0.5	39.7 ± 0.8	37.3 ± 0.8	38.5 ± 0.8
n-3	34.5 ± 0.2	29.5 ± 1.2	25.3 ± 1.1	27.4 ± 1.2
n-6	4.4 ± 0.0	10.2 ± 0.3	12.0 ± 0.3	11.1 ± 0.3
n-3/n-6	7.9	2.9	2.1	2.5

Poly-unsaturated fatty acids (PUFAs) represented the most dominant class of fatty acids in sprats (38.9% of total fatty acids), followed by mono-unsaturated fatty acids (MUFAs) with 31.8% and saturated fatty acids (SFAs) with 29.3% (Table 1). In the PUFAs fraction, unsaturated fatty acids with a double bond after the third carbon atom (n-3) were present more than unsaturated fatty acids with a double bond after the sixth carbon atom (n-6),

with an n-3/n-6 ratio of 7.9 on average. The profile of fatty acids in sprats is comparable to that in rainbow trout PUFAs 38.5%, MUFAs 37.9%, and SFAs 22.8% (Table 1). In rainbow trout samples, C16:0, C18:1, and docosahexaenoic acid (DHA, C22:6n-3) were dominant. The linoleic acid (18:2n-6) content in all rainbow trout analyzed ranged from 7.0 to 11.9 g / 100 g lipid. Linoleic acid was the major n-6 PUFA in rainbow trout and is responsible for the n-3/n-6 PUFA ratio on average 2.5, which correlates well with earlier research results by Blanchett *et al.* [82].

A descriptive sensory analysis of gutted and steam-baked sprats demonstrates that fish form different catching seasons grouped together and the main perceived differences were in hardness (spring sprats being the hardest), and sweetness and bone separation (autumn and winter sprats were sweeter and had better bone separation) (Publication I, Figure 2). These sensory characteristics, which are also desirable in producing spice-cured sprats, correlate well with fat content. Fat content of sprat is especially important to the sensory properties when products are minimally processed and lightly preserved, as in spice-cured sprat products. It has been suggested that fat oxidation during ripening generates the typical flavor of cured fish [33]. Fat content has been found to correlate with the seasonal catch of the fish and enzyme activity [5]. Fatty, autumn and winter sprat most probably contain more enzymes which answer for proteolysis, and thus fat content of sprat is related not only to nice succulent mouthfeel, but also to formation of desired flavor compounds.

5.2 CHEMICAL, MICROBIAL AND SENSORY CHANGES IN SPICE-CURED SPRATS

Studies on the seasonal variation of Baltic sprat (Publication I), together with literature data and industrial experience have shown that sprat has the best quality for curing during autumn. Below, the results of model experiments (Publication II, Publication III) that describe the chemical, sensory and microbiological changes that occur during curing along with the influence of freezing-thawing and package material are summarized. The model curing process was carried out in subsequent years with only the autumn catch — in four variations: (I) from fresh fish using glass jars; (II) from frozen-thawed fish using glass jars; (III) from fresh fish using plastic jars; and (IV) from frozen-thawed fish using plastic jars. The quality parameters of these fish were analyzed over the shelf life of these products, at weeks 0, 1, 4, 8, 10, and 12.

5.2.1 *Changes in the chemical composition of sprats during curing*

After sprats were covered with brine, the process of salting was completed after 4 weeks at which time the NaCl content in fish and brine equalibriated at a level of 6% in the fish (Publication II). Salt penetration into the fish may affect the water content due to the removal of water from the fish and replacement of water with salts during curing. Another factor which can influence the water content of spice-cured sprats is the freezing-thawing pretreatment, because muscle cells may be disrupted by ice crystals, thus allowing their contents to leak out. In cured sprats, the water content was stable throughout the 10 weeks of study; No statistically significant differences caused by freezing-thawing or salt-

water replacement, during the ripening or storage were observed (Publication II, Table 1).

5.2.1.1 *Protein content and pH*

Loss of proteins due to the hydrolysis and extraction into brine is one of the key elements in the process of fish curing. The protein content in the fresh fish fillet was 13.8 $\pm$ 0.6 $g \cdot g^{-1}$ and 14.9 $\pm$ 0.33 $g \cdot g^{-1}$ for 2009 and 2010, respectively, and decreased throughout the entire ripening period by about 30% (Publication II, Table 1). The pH in both the fish fillet and brine decreased slightly throughout the entire ripening period (Publication II, Figure 1). The pH in the brine was lower than in the fish, which may be due to the difference in buffering capacity of brine and fillet. There was a tendency that the brine pH was slightly lower in glass jars than in plastic jars and that brine pH was slightly lower in samples made from frozen-thawed fish than in samples made from fresh fish (Publication II, Figure 1). This was observed in batches from both 2009 and 2010. The decrease in pH during ripening can be related to peculiarities of protein hydrolysis and acid formation in glycolytic processes.

5.2.1.2 *Free amino acid content*

Free amino acid FAA content is one of the most widely used parameters for describing the extent of fish curing. The total content of FAAs increased during the ripening process of spice-cured sprats (Table 2). During the first 8 weeks of ripening there was a tendency that the total FAA content increased slower in spice-cured sprat samples made from fresh fish, than those made from frozen-thawed fish. The FAA content also increased faster in samples contained in plastic jars compared with glass jars (Figure 5). The basic/acidic amino acid ratio (B/A) was equal to 3.5 in fresh fish, and decreased with freezing-thawing down to 2.5 (Figure 6). During ripening, the B/A further increased to a level of 1.3-1.5 at week 4, at which point the desired qualities of spice-cured sprats were obtained. Samples made from fresh fish and packed into glass jars maintained a higher B/A than other samples until the end of the ripening experiment. The B/A of other samples decreased and the optimum quality was not preserved. Spice-cured sprats in plastic packages and from frozen-thawed fish ripened faster than other all other samples.

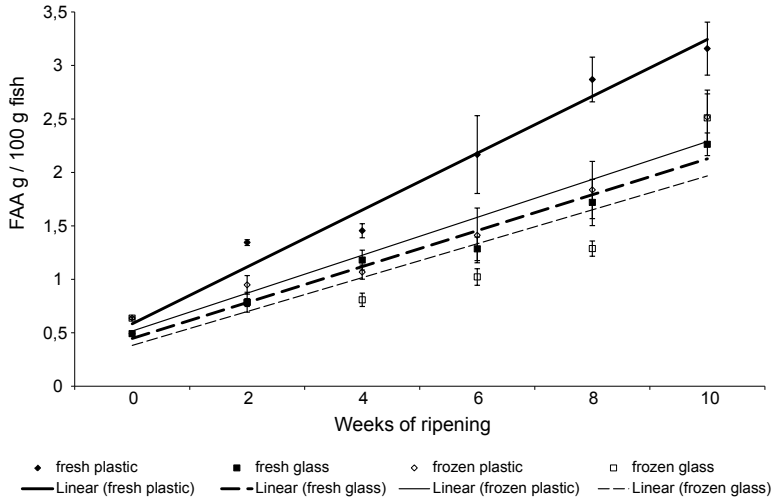

Figure 5 – FAA content of spice-cured sprats in glass and in plastic package made from fresh and frozen-thawed fish. Number 0 is fresh fish and numbers 2-10 show the week of ripening. The results are given for the 2010 catch.

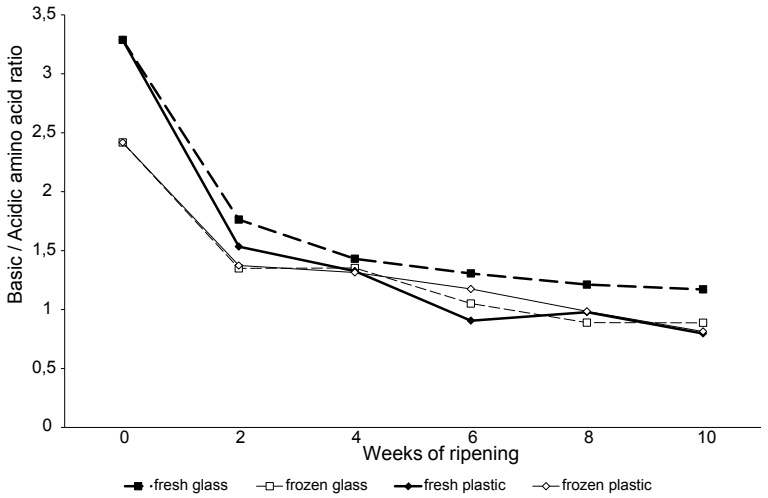

Figure 6 – B/A of spice-cured sprats in glass and in plastic package made from fresh and frozen-thawed fish. Number 0 is fresh fish and numbers 2-10 show the week of ripening. The results are given for the 2010 catch.

Table 2 – FAA content (mg / 100 g) of fresh sprat, frozen-thawed sprat, and spice-cured sprats at week 10 of ripening. Letters A and B stand for two different batches (A – 2009, B – 2010). Letters a, b, c and d stand for significant differences between fresh and frozen-thawed fish; spice-cured sprats made from fresh fish and packed into glass and plastic jars; spice-cured sprats made from frozen-thawed fish and packed into glass and plastic jars.

Amino acid	Fresh						Frozen-thawed					
			Glass		Plastic				Glass		Plastic	
	A Fresh	B Fresh	A 10 wks	B 10 wks	A 10 wks	B 10 wks	A Frozen-thawed	B Frozen-thawed	A 10 wks	B 10 wks	A 10 wks	B 10 wks
Ala	56	65	97	138	137	250	42	70	82	165	135	174
Arg	10	15	135	186	71	14	13	23	112	91	75	95
Asn	4	1	1	2	2	20	2	1	1	1	1	3
Asp	6	6	106	139	148	202	8	22	90	201	154	213
Cys	0	3	6	7	8	8	0	1	6	11	9	8
Gln	7	13	42	240	89	400	2	9	31	188	61	143
Glu	17	19	97	108	132	175	15	41	89	199	147	206
Gly	21	25	41	63	60	128	18	25	30	67	52	75
His	114	143	72	122	95	159	111	203	52	206	74	178
Ile	9	9	67	104	87	157	8	16	56	113	93	115
Leu	15	24	135	196	172	279	15	31	118	222	193	227
Lys	33	34	141	193	190	306	25	53	119	241	197	236
Met	8	12	59	101	60	135	6	13	53	108	81	114
Phe	9	13	71	128	90	180	8	15	63	127	103	149
Pro	9	11	50	71	71	108	8	15	43	81	69	88
Ser	13	17	62	96	86	93	14	32	50	112	84	108
Thr	13	17	62	86	82	131	11	20	52	93	89	97
Trp	6	34	23	49	27	63	3	5	20	40	31	49
Tyr	10	13	64	113	80	147	9	15	56	110	91	99
Val	14	19	84	120	109	201	13	25	68	134	114	139
Σ	374^{b}	492^{c}	1416^{a}	2263^{c}	1797^{b}	3157^{d}	328^{a}	636^{d}	1190^{a}	2510^{c}	1852^{b}	2517^{c}
B/A	3.3^{c}	3.3^{c}	1.1^{b}	1.2^{c}	0.8^{a}	0.8^{a}	3.2^{a}	2.4^{b}	1.0^{d}	0.9^{c}	0.7^{a}	0.8^{b}

5.2.1.3 *Lipid and fatty acid content*

The average lipid content of the fresh fish and ripened samples was determined using the Soxhlet method. The lipid content of fresh fish was found to be 9.4 $\pm$ 1.1 and 12.1 $\pm$ 1.7, for the 2009 and 2010 catches, respectively, and the lipid content did not change significantly during the 10 weeks of ripening (Publication II, Table 1). The packaging method or freeze-thawing did not affect the lipid content.

The fat deposits in sprat contain high levels of mono- and polyunsaturated fatty acids (MUFAs and PUFAs), which are excellent from a nutritional point of view (Table 1), however, these fats have a tendency to oxidize during processing and curing of fish products. Spice-cured sprats have a long shelf-life compared to fresh fish and thus it is of interest to evaluate the fatty acid profile of the product, and possible effects introduced by freezing-thawing and the use of plastic packaging. The fatty acid composition of spice-cured sprats was stable during processing, and only a slight increase in MUFA 18:1 and decrease in total PUFAs was observed in frozen-thawed ripened sprats. The effect can be explained by the action of prooxidants, such as metals and myglobin, and hemoglobin in sprat blood [83, 84], and enzymes released during the freeze-thaw cycle which are responsible for fatty acid metabolism [85].

5.2.2 *Textural changes in spice-cured sprats*

Texture of a product is an important attribute. The curing of fish changes the relatively hard structure of fresh fish into a softer and palatable fish product. With very small fish, such as sprat, it is impossible to cut sample pieces from fillets with definite fiber orientation, dimensions, and due to the variation of each individual, the variation in texture measurements is very large. Thus, a small deformation rheological method, described by Badii and Howell [75] was chosen to evaluate the textural properties of spice-cured sprats.

5.2.3 *Small deformation rheological test*

Viscoelastic measurements of spice-cured sprats muscle homogenate are presented for the samples ripened for 10 weeks made from fresh fish in plastic jars (Publication II, Figure 3a) and in glass jars (Publication II, Figure 3b). In all cases, prior to heating, the storage modules (G') was found to be greater than the loss modules (G''), indicating that the homogenate had a gel-like structure (Publication II, Figure 3, Table 4). Saeed and Howell [86] show with their small deformation rheological test that the main protein affected in frozen-thawed fish is myosin, followed by actin, and that lipid oxidation may contribute to protein aggregation, which results in tougher fish fillets and higher elastic modules values. The storage modules (G') and loss modules (G'') values at week 8 ripened sprats homogenates were higher in samples in glass jars than in plastic jars, for both samples made from fresh and frozen-thawed fish (Publication II, Table 3). This indicates that samples ripened at a faster rate in plastic package. There is a tendency that samples made from frozen-thawed sprats have higher storage modules (G') and loss modules (G'') values

at 8 weeks of ripening, than samples made from fresh sprats (Publication II). This finding supports the theory that frozen-thawed samples do not always acquire similar texture properties as samples made from fresh fish. This can be explained by the influence of freezing on the decrease in the intestinal or microbial activity of fish [87, 88], and the decrease in water-holding capacity [89, 90].

5.2.4 *Microbial activity in spice-cured sprats*

During the first four weeks of curing, the NaCl equilibrated at 6% in the fish and brine, which, together with low temperature 4℃, selectively inhibited microbial growth. In addition, the spice mix used for curing may selectively affect the growth of microorganisms present in sprats. Microbiota contribute to the flavor development, proteolysis, ripening, but also promote spoilage, and explain the faster hydrolysis observed in plastic containers compared with glass jars. Plate counts of total bacterial load and identification of dominating species was performed in order to evaluate the potential effect of microbiota on the quality parameters developed during the curing of sprats. In addition these measurements allow one to identify differences in bacterial growth with the use of both freezing-thawing and plastic packaging.

5.2.4.1 *Plate counts*

Plate counts on MRS and plate count agar media depended on the catch (both yearly and seasonal) and were on average one magnitude lower for frozen-thawed fish compared to fresh fish. The plate counts were low and remained in the range of 10^2 to 10^3 CFU·g^{-1}. The plate counts of frozen fish were reduced compared to fresh fish raw material, an effect also observed in other studies, *e.g.* [91]. After the 4^{th} week of curing it was observed that products in plastic jars had higher numbers of colony forming bacteria, regardless of whether fresh or frozen-thawed fish was used in the curing process. During weeks 8-12 of curing, plate counts of spice-cured sprats in plastic jars reached higher values 10^6 to 10^7, compared with samples ripened in glass 10^6 CFU·g^{-1}.

5.2.4.2 *Species*

Isolates from fresh and frozen sprat predominantly belonged to the genus *Carnobacterium* (Publication IV, Table 1 and Table 2). In addition isolates of *Brochothrix thermospacta*, *Enterococcus* spp., *Hafnia* spp. and *Vagococcus* spp. were found in fresh fish (total 10^3 CFU·g^{-1}). Microbial counts of frozen-thawed fish were lower (total 10^2 CFU·g^{-1}). In addition to the species found in fresh sprats, *Enterobacter* spp. and *Lactobacillus sakei* were both found, probably due to their resistance to freezing-thawing. *Lactobacillus sakei/curvatus*, *Aerococcus viridians*, and *Brochothrix thermospacta* reached $>10^6$ CFU·g^{-1} during curing; Levels that may enhance both sensory properties and the spoilage of the product [63, 92–94].

There was a clear difference in the patterns of species that were found in samples made from fresh and frozen-thawed fish, and those cured in glass and in plastic jars. The patterns of species showed that *Brochothrix thermospacta*, which was abundant in fresh

sprats was growing in all spice-cured sprat samples made from fresh and frozen-thawed fish and packed into glass and plastic jars (Publication IV, Table 1 and Table 2).

5.2.5 *Sensory properties of spice-cured sprats*

The most important attributes which describe spice-cured sprat ripening were: hardness, moistness, sour taste, rancid flavor, and general spiciness (Figure 7). General spiciness and moistness of samples had a tendency to rise with ripening time, this was noted for all samples, regardless of catch, pretreatment, or package. Hardness of samples correlated well with ripening time, less ripened samples were harder and more ripened samples were softer (Publication II, Figure 4). Sour, and rancid flavors developed more rapidly in spice-cured sprat samples made from frozen-thawed fish than samples made from fresh fish, and they also had a tendency to be harder (Figure 7). Spice-cured sprat samples packed into plastic packages also had a tendency to be more sour and their rancid flavors developed more rapidly than samples packed into glass jars (Publication II, Figure 4).

5.2.6 *Consumer acceptance of spice-cured sprats*

The aim of the consumer acceptance study was to describe and evaluate the competitiveness of Estonian spice-cured sprat products in the local market (Estonia) and in a new market (Thailand). The central location trials (CLTs) were conducted in Bangkok, Thailand, and in Tallinn, Estonia, with 106 and 111 consumers respectively (Publication III). Spice-cured sprat sample A was saltiest, sample B had the lowest overall spiciness, and sample C had highest pepper flavor and sour taste intensity. The highest correlation coefficients were detected between "overall liking" and flavor (0.71), appearance (0.69), and fish flavor (0.61) in Estonia; and appearance (0.68), odor (0.58), and fish flavor (0.57) in Thailand. Differences in the consumer appreciation of fish flavor and sourness divided Estonian and Thai consumers into clusters (Publication III, Figure 1). In Estonia, one cluster of consumers liked the traditional (sample A) and lightly spiced (sample B) sprat products, while the other cluster liked the traditional (sample A) and the marinated (sample C) sprat products. In Thailand all samples scored low, but manual clustering indicated that marinated (sample C) sprat products were most acceptable.

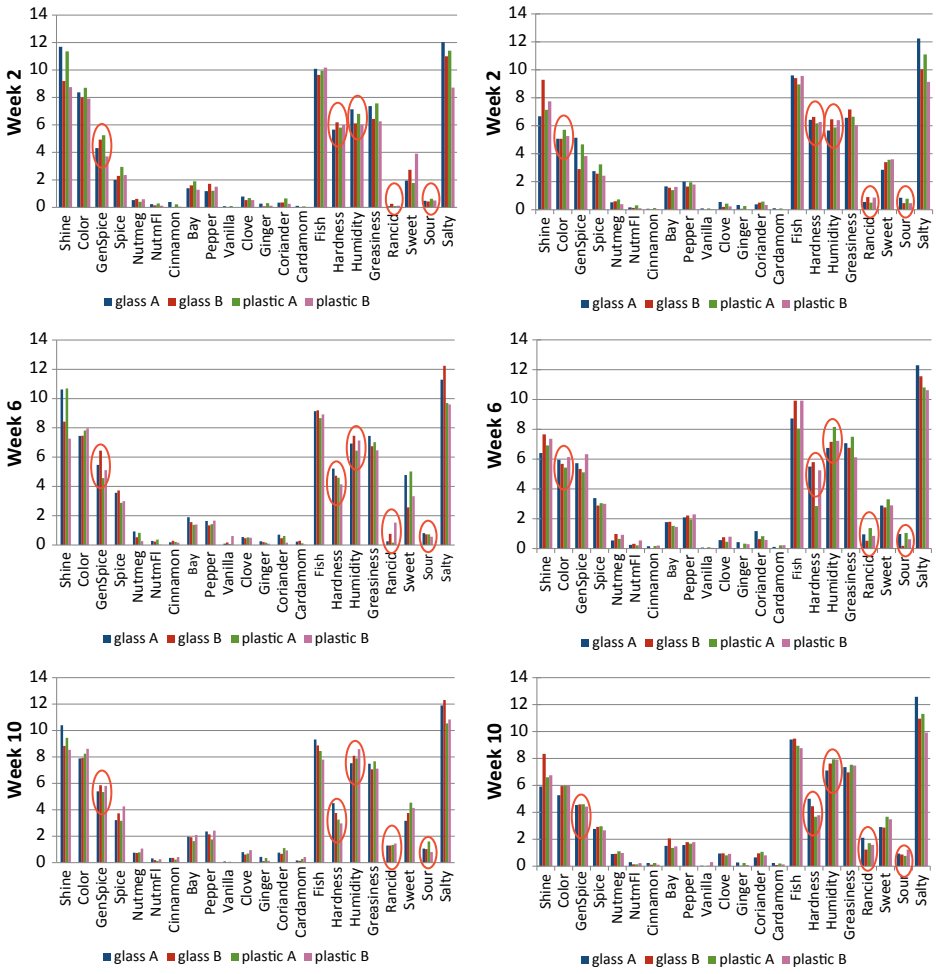

Figure 7 – Sensory properties of spice-cured sprats made from fresh and frozen-thawed fish, week 2, 6 and 10. Letters A and B stand for two different catches (A – 2009, B – 2010).

6

DISCUSSION

The results of this dissertation are discussed in the following five sections.

6.1 RIPENING OF SPICE-CURED SPRATS

The ripening of spice-cured sprats is a process which transforms the raw sprats into a delicious and succulent ready-to-eat fish product. Spoilage starts from the moment the quality of the product starts to decrease.

The ripening of fish products has been described for several different fish species such as herring, anchovies, and sardine [9, 12, 34, 40]. They have all found that the process of ripening is similar, but the key descriptors and scales vary according to fish species and the production technology used produce these products.

Salting is the first process that initializes the ripening of fish and gives a pleasant salty taste, inhibits growth of undesired microbiota, and lengthens the shelf-life of the product. Less and less salt is used to cure fish products, because of both the availability of alternative shelf-life prolonging treatments (temperature control, packaging materials and environments) and consumer preferences for less saltier products. However in the case of spice-cured sprat products the salting process remains traditional and uses 23% NaCl brine, which results in an end product that contains about 6% NaCl content. This concentration is lower than the amount required to denature fish proteins [15] as well as inhibit the growth of salt tolerant bacteria.

One question that remains is: What is the role of muscle proteases, and intestinal and microbial proteases during sprat ripening? Earlier studies have demonstrated that intestinal proteases are important for sprat curing [5], however the role of microorganisms during ripening and spoilage of products remains unclear.

The current study shows that FAA content and particularly basic/acidic amino acid ratio (B/A) is useful as a quality indicator to monitor the ripening of spice-cured sprats. The FAA content of spice-cured sprats increased throughout the ripening while B/A decreased. B/A [34] has been used as a curing parameter for other studies of fish curing [9, 12, 39, 40]. It has been suggested that the cured fish is considered ripe, with regards to sensory analysis, when B/A is around 1.0 [9, 12, 34, 35, 39, 40]. The FAA content of spice-cured sprats increased throughout ripening and obtained optimum sensory properties while the B/A was around 1.0 (Figure 6). At that point the hardness of the spice-cured sprats had decreased and moistness had increased to a point where a nice succulent mouthfeel was achieved, general spiciness of fish was highest, and sour and rancid notes did not

dominate (Figure 7). The decrease in B/A to <1 in our work correlates with a decrease in sensory properties, and particularly with regards to the increase in sour and rancid flavors.

Empirical evidnece and experience dictates that to produce high quality spice-cured sprat products, one requires fresh raw material, oxygen tight package materials, and preservation at 2-4℃. Today, additional quality issues have arisen with technology modifications, *e.g.* freezing-thawing raw material and different packaging into oxygen transparent materials.

In this study the chemical, microbiological and physical processes that occur during sprat curing in the traditional process model were studied in comparison with modern technological trends including the use of frozen fish and in some occasions oxygen permeable packaging materials.

6.2 EFFECT OF FREEZING-THAWING ON THE RAW MATERIAL

With pelagic fish species (*e.g.*, sprat) it is inevitable that large amounts of fish are harvested periodically and delivered to fish production facilities. It is thus prohibitively difficult and ineffective to process the entire batch into products. Thus, freezing of raw material is a good option, however, freezing may influence the quality of the raw material and end products [86–90]. The effects of freezing-thawing on fish quality during the curing process were reflected by a slower rate of free amino acid formation (Publication II), which might be due to the lower solubility of frozen proteins [95–97] or sensitivity of proteolytic enzymes to freezing-thawing. From this it can be concluded that freezing-thawing of sprats will affect the proteolysis and ripening process of spice-cured sprats as well as their shelf-life.

Products prepared from frozen-thawed fish had a number of undesired properties including a sour taste and rancid flavor, which were pronounced already at week 2 (Figure 7). This tendency was observed throughout the entire observation period. Judging the effect of this is difficult because proteolysis was found to be slower in freeze-thawed sprats compared with fresh fish. Also, the contamination and growth at initial stages of curing was lower in freeze-thawed sprats.

6.3 PACKAGE MATERIAL

Packaging material may influence the properties of the product by changing the environment the food is exposed to (*e.g.*, aerobic, anaerobic, modified atmosphere packaging (MAP), permeability of gas). Metal and glass jars provide an ideal anaerobic condition important for spice-cured sprats. However, plastic jars are lighter and more convenient, however, they do allow more oxygen to permeate. According to sensory analysis and the B/A values measured spice-cured sprats made from fresh fish and packed into glass package had the highest and most stable quality after optimal quality was obtained at week four (Figure 6). The sensory properties and B/A of spice-cured sprats in plastic containers and from frozen-thawed fish showed a loss of the optimum quality by week 8. From a sensory quality point of view, the decrease in B/A shows that the relative in-

crease of acidic FAAs may cause the sour taste of the spice-cured sprats. The sour taste is the main indicator of spoilage, thus it is important that the build up of acidic FAAs is retarded. On the other hand, the increase of FAAs is also an indicator of proteolysis, which lowers the hardness of the fish, and thus without ripening it is not possible. Amino acids form faster in plastic containers compared with glass containers with a similar geometry. This can be explained by the permeability of plastic packages to gases, especially oxygen, which can promote microbial processes (Publication IV).

6.4 MICROBIOTA

The number of microorganism in raw material was very low, only about 10^3 CFU·g^{-1} and freezing-thawing decreased this number down to 10^2 CFU·g^{-1}. The number of bacteria in food typically required to influence the sensory properties of a product is 10^6 to 10^7 CFU·g^{-1} [63, 92–94]. In spice-cured sprats during ripening, three species were found to have plate counts over 10^6 CFU·g^{-1}: *Brochothrix thermospacta*, *Lactobacillus sakei*, and *Aerococcus viridians* (Publication IV). Those species were present in very low numbers: in fresh fish N $<$ 100 CFU·g^{-1} and freeze-thawed fish N $<$ 10 CFU·g^{-1}. When taking into account the low ripening temperature, it takes 4-8 weeks to achieve the million limit.

Different bacteria were found to dominate in different samples and experimental points. This can be explained by microbial heterogeneity of the sprat microbiota which is well demonstrated by the large number of fingerprints presented in Publication IV, Figure 1. Similar fingerprints in raw fish and cured sprats from same material were found only on a few occasions.

It would be interesting to further study the effect of those microorganisms isolated from sprats during the ripening and spoilage of cured sprats. Inoculation of sprats with one or more of these organisms might have both positive and negative effects. One of the positive effects could be an acceleration of ripening while preventing the growth of spoilage microorganisms. An increase in spoilage may be considered as a negative effect. According to the literature *Brochothrix thermospacta* is known as a moderate spoilage organism, and oxygen availability increases the concentration of metabolites produced by this species [93]. *Aerococcus viridians* was not detected in spice-cured sprats made from frozen-thawed fish, which suggests a negative effect of freezing on the cultivability and viability of these bacteria (Publication IV).

Lactobacillus sakei plate counts increased in spice-cured sprats during storage. *Lactobacillus sakei* is known to have a high salt tolerance and it produces H_2S and lactic acid [50, 58, 98–101] and has been reported to be a major spoilage bacteria in chilled seafood [63] and meat products [98]. This work presented here supports the view that *Lactobacillus sakei* promotes spoilage more than ripening. This, however, does not exclude the possibility that some bacteria may promoting both ripening and biopreservation. Alternative preservation strategies could be adopted to prolong sensory shelf-life. Storage at a subzero temperatures (-2℃), may also prevent the growth of microorganisms and prolong the storage time, or alternatively biopreservation could be applied using microorganisms that are antagonistic to the spoilage bacteria [102–104].

6.5 SENSORY PROPERTIES AND CONSUMER ACCEPTANCE

Another line of study undertaken in this doctoral work was to assess the consumer acceptance of spice-cured sprat products. This is important because the descriptive analysis used in the laboratory by a trained panel does not necessarily provide accurate information about consumer acceptance of a given product.

It took four weeks of ripening before the sprats obtained sour and rancid flavors (Publication IV, Table 1 and 2). The rancid flavor may be related to protein or fat hydrolysis, or to conversions that occur in the spice mix. To what extent this sour taste and rancid flavor is accepted by consumers is another question.

There was significant variance in the chemical and biological parameters between batches made from fresh and frozen-thawed fish, and those of packed in glass jars and plastic containers. Clear trends were observed that show correlations of sensory data with ripening time while only slight differences in sour, rancid, hardness, and general spiciness were observed in the same experimental time point. One of the very crucial factors which added variability in the descriptive test results was the spice-mixture distribution in the jars. In the model process, the spice-mixture was placed on the bottom of jars and the flavors from the spice-mixture did not distribute evenly throughout the product, and caused a high variability in sensory data. From this it can be concluded that flavors from the spice mixture did not diffuse evenly throughout the sprat flesh in the jars, and in future studies it is recommended that one mix the fish in the jars to ensure an even coating of spice-mixture. This may also be required to obtain a product with a stable flavor profile.

Spice-cured sprat samples packed into plastic jars had lower hardness, and higher sourness and rancidity than spice-cured sprats in glass jars at week 8. This can be related to the activity of microorganisms. Spice-cured samples made from frozen-thawed fish had a tendency to be harder, and sour and rancid flavor developed more rapidly than in samples made from fresh fish (Publication II, Figure 4). The spice-cured sprats obtained optimal sensory properties (hardness, moistness, and general spiciness) during 4 weeks of ripening. Importantly, the microbial concentrations remained too low to affect the sensory properties throughout the entire ripening process. Thus, we can conclude that the role of microorganisms in the ripening of spice-cured sprats is small, but they do affect the shelf-life of this product (Publication IV, Table 1 and 2).

Spice-cured sprat products are popular in the local and Eastern European market, however, new markets are of interest. Thus, a comparative acceptance study of spice-cured sprat products in Estonia and in Thailand was conducted. The most characteristic attributes which differentiate spice-cured sprat products and consumer acceptance were saltiness, fishiness, and sourness (Publication III, Figure 1). Spice-cured sprat products were not enjoyed as much in Thailand compared with Estonia (Publication III, Table 6). Because these spice-cured sprat products are completely new to Thai consumers, the low scores are valuable information. This informs the producer that these consumers will probably not repurchase this product. This trend has been observed in consumer food choice studies with product experiences that are new to consumers [105]. The consumer acceptance study in this dissertation shows that spice-cured sprat products in general can

be accepted by Thai consumers, especially as part of meals, if further flavor development is carried out with the products.

Consumer acceptance of spice-cured sprat products came with recommendations to reduce the salt content. In addition, products imported to Thailand should have increased spiciness and acidity. With regards to technological aspects, a lower salt content may influence the properties of the product by promoting the growth of undesired microbiota. However, a higher acidity and lower pH will suppress the growth of certain microorganisms but will enable the growth of certain acid tolerant microorganisms. In creating less salty and more acidic spice-cured sprat products, biopreservation is one possible solution. The salt tolerant microorganisms isolated in this study (*Brochothrix thermospacta*) could be used in this development process. Another potential development of spice-cured sprats would be an improvement in technology to fillet spice-cured sprats. As the current study shows, spice-cured sprats made from frozen-thawed fish does not have the same quality properties as products made from fresh fish. Similar technology used in maatjes herring production could be adopted to fillet spice-cured sprats; All sprats would be spice-cured freshly, then filleted at optimum ripening and frozen until further use.

SUMMARY

7

CONCLUSIONS

Six conclusions result from this dissertation.

I While spice-cured sprat products are preserved in salt, proteolytic and microbial processes continue over the duration of their shelf-life. This causes variation in both the sensory and quality properties of the product as a function of shelf life.

II The ripening process of spice-cured sprats begins with salting-in, followed by proteolysis due to the intestine and muscle proteases. The shelf-life of the product may be influenced by salt tolerant species of fish microbiota.

III The ratio of basic and acidic amino acids was the most useful ripening indicator and was found to correlate well with the sensory properties of spice-cured sprats.

IV Freezing followed by thawing of fish prior to curing decreased the activity of enzymatic and microbiological processes during sprat ripening.

V Curing sprats in plastic packaging material enhances the growth of microbiota, changes the composition of microbiota, and raises the sourness and rancidity compared with ripening in glass jars. These differences are attributed to the increased permeability of oxygen through the plastic containers.

VI Consumer acceptance studies of spice-cured sprat products provided recommendations to reduce the salt content. In addition, products to be exported to Thailand should have increased spiciness and acidity.

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APPENDICES

PUBLICATION I

Timberg L, Koppel K, Kuldjärv R, Paalme T

Sensory and chemical properties of Baltic sprat (*Sprattus sprattus balticus*) and Baltic herring (*Clupea harengus membras*) in different catching seasons

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Sensory and Chemical Properties of Baltic Sprat (*Sprattus sprattus balticus)* and Baltic Herring (*Clupea harengus membras*) in Different Catching Seasons

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Abstract. Baltic sprat (*Sprattus sprattus balticus*) and Baltic herring (*Clupea harengus membras L*) are two of the most caught fish species among the Estonian seacoast fishermen, and therefore it is important to understand catching season effects on sprat and herring sensory and nutritional quality. The aim of this study was to measure and compare sensory and chemical variability of Baltic sprat and Baltic herring during different catching seasons. Batches of Baltic sprat and Baltic herring were caught from different locations in Estonian coastal waters from February 2008 until April 2009. Water content, protein content, lipid and ash content were measured. Descriptive sensory evaluation of steamed fish was conducted and results analyzed using Partial Least Squares Regression. The results suggested differentiation possibilities between fish from different seasons. The variations lie in fat and water contents, hardness, characteristic flavor and sweetness of the fish flesh.

Key words: Baltic herring (*Clupea harengus membras L*), Baltic sprat (*Sprattus sprattus balticus*), descriptive sensory evaluation

INTRODUCTION

Baltic sprat and Baltic herring and dishes of these fish have been eaten by people living around the Baltic Sea for centuries. Many different technologies like smoking, spicing, salting, marinating, fermenting etc. are used to produce fish products from Baltic sprat and Baltic herring for the Baltic countries and for the Eastern European markets. Some of these product types are very sensitive to fish quality and others are less, because of strong flavor and structure additives.

Nutritional composition of Baltic sprat caught from coastal waters in Estonia has been previously monitored and reported by Krosing and Veldre (1973). Baltic herring composition has been monitored and reported by several authors (Kolakowska et al., 1992; Szlinder-Richert et al., 2010). Baltic sprat and Baltic herring are under strict surveillance when it comes to dioxins and other contaminants (Vuorinen et al., 2002; Simm, et al., 2006; Szlinder-Richert et al., 2009), but until now the composition and sensory quality of the Baltic sprat and Baltic herring from gulf of Riga and Finland have not been thoroughly studied. There is clear evidence that the ecological state of

health of the Baltic Sea has changed remarkably during past decades, and it also influences the environment of the fish living in the Baltic Sea (Lankov et al., 2010; Raid et al., 2010; Ojaveer et al., 2011).

Sensory analysis gives a holistic and integrated picture of the fish whereas instrumental methods generally measure only one specific compound or a set of attributes related to one set of properties (Nielsen, 1997). Sensory analysis can be more accurately interpreted by using various data from instrumental analysis. Partial least squares technique is used to show relationship between sensory attributes and chemical variables. Baltic sprat and Baltic herring composition is known to vary the most in lipid and water content (Krosing & Veldre, 1973; Kolakowska et al., 1992; Szlinder-Richert et al., 2010). The variation in the chemical composition of Baltic sprat and Baltic herring is related to nutrition, catching season, fish size, seasonal and sexual variations. Variation in chemical composition might lead to changes in sensory attributes, including flavor, aroma, texture, and visual appearance which control the acceptability of fish as food (Flick & Martin, 1992). The aim of the current work was to evaluate the sensory properties and their relations to lipid content, water content and protein content of Baltic sprat and Baltic herring.

MATERIALS AND METHODS

Samples

Twenty-six samples of Baltic sprat and 33 samples of Baltic herring were caught from different locations in Estonia's coastal waters (Gulf of Riga and Finland) from February 2008 until March 2009. Samples were taken only from spring-spawn fish populations. Samples of fish (about 2 kg) were immediately frozen after landing and stored at −18°C. The fish was analyzed during two months followed to catching. The frozen fish samples were thawed at 4°C 48 hours before analysis. Thawed fish were de-headed, gutted, de-boned and washed. Samples for sensory analysis were packed by two in aluminum foil, steamed for 10 minutes at 65°C, and served immediately.

Composition analysis

Samples for composition analysis (minimum 1 kg) were minced twice and kept at 4°C until analyzed within the same day. All measurements were carried out in triplicate. Water content of the fish samples was measured using a halogen analyzer (HR 83, Mettler Toledo, Switzerland). The protein content of the fish samples was measured by Kjeldhal method (Velp Scientifica UDK 142, Italy). The lipid content of fish was measured by Soxhlet method (Velp Scientifica SER 148 Solvent Extractor, Italy). All necessary reagents were purchased from Sigma-Aldrich, Germany.

Sensory Analysis

Descriptive sensory analysis was performed by 5 trained panelists. Sensory analysis was conducted in a laboratory equipped with individual booths (ISO 8589–1988). The panelists were trained during five one-hour sessions to evaluate the appearance (skin color, meat color, gapping, broken skin, shape), flavor (off-flavor, characteristic flavor, off-aroma, characteristic aroma, sweetness), and texture (hardness, bone separation, cohesiveness, moistness, greasiness) attributes. All of the

panelists had at least 50 hours of previous testing experience in descriptive evaluation of fish products. The samples were coded with random three-digit numbers and evaluated in two repetitions on a 9-point numerical scale, anchored at both ends. The evaluations took place shortly after the samples were steamed. Unsalted crackers and purified water were available for palate cleansing.

Statistical Analysis

The data was analyzed using the Unscrambler 9.8 (Camo Software, Norway). Partial Least Squares (PLS1) regression was used to plot average sensory scores and composition data. The data matrix included humidity content and sensory attribute scores for data regression. XLSTAT (2009, Addinsoft, France) was used to calculate the correlations between sensory and chemical or biological properties (Pearson, P = 0.05).

RESULTS AND DISCUSSION

Baltic sprat

Composition of Baltic sprat remained in range 57–73% water, 15–17% protein, 10–24% lipid, and 2–4% ash (Fig. 1). Krosing and Veldre (1973) showed in their studies that Baltic sprat composition was 66–80% water, 15–17% protein and 3–18% lipid. Research results showed an inverse relationship between water and lipid - the lower the water content the higher the lipid content and vice versa, which has also been observed by other researchers (Rehbein & Oehlenschläger, 2009). Lipid content of the fish increased from 13 ± 1.6% in spring to maximum values in October and November, on average 22 ± 3%. As the lipid content increased, the water content decreased in

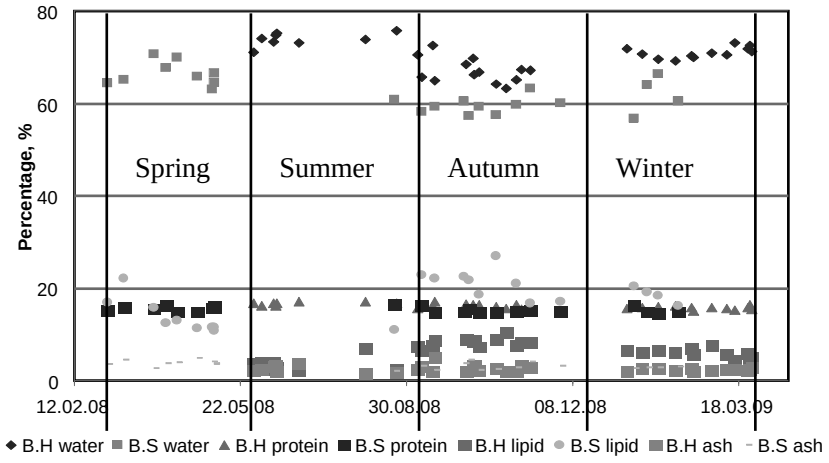

Figure 1. Baltic sprat & Baltic herring composition, where B.H water – Baltic herring water%, B.S water – Baltic sprat water%, B.H protein – Baltic herring protein%, B.S protein – Baltic sprat protein%, B.H lipid – Baltic herring lipid%, B.S lipid – Baltic sprat lipid%, B.H ash – Baltic herring ash%, B.S ash – Baltic sprat ash%.

autumn. Protein content was quite stabile, in winter on average 15.1 ± 0.6%, in autumn on average 15.3 ± 0.7%, in spring on average 15.5 ± 0.5%, and according to this study there was no distinct connection between the season and protein content of Baltic sprat and Baltic herring.

Sensory and composition measurements

Autumn samples had the lowest water content and spring samples had the highest water content (Fig. 2). There was a strong negative correlation between water and lipid content of sprats (R = -0.82, *P* = 0.05). PLS component 1 explained the hardness versus the humidity; winter and autumn samples were harder than spring and summer sprats. Autumn and winter sprats were sweeter than spring sprats.

The lipid content of Baltic sprat and greasiness attribute were significantly correlated (R = 0.48). However, no correlation was detected between lipid content, flavor, and aroma. Baltic sprat lipid content was relatively high, over 10%, even in the spring and summer periods, when it contained the least fat. This may explain the absence of seasonal variation in flavor and aroma intensity.

Baltic sprat samples grouped into three distinctive groups: samples from January and February (winter) made up the first group; samples from April and May (spring) made up the second group; and samples from late August to November (autumn) made up the third group (Fig. 2). The three groups suggest that there is a difference in sensory properties between winter, spring and autumn Baltic sprats.

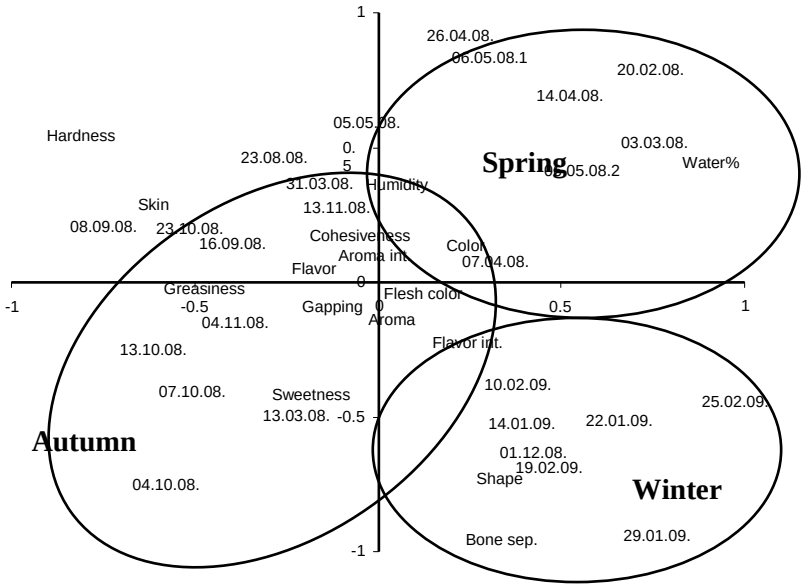

Figure 2. PLS analysis of Baltic sprat sensory attributes, sprat batches, and water content (batch code DD.MM.YY); x-explained 23% (water%), 21% (sensory attributes); y-explained 51% (water%), 15% (sensory attributes). The circles represent three seasonal groups.

Baltic herring

Composition of Baltic herring was 65–75% water, 15–17% protein, 3–10% lipid, and 2–3% ash (Fig. 1). There was a similar inverse correlation between the water and lipid content as was seen with Baltic sprats. Baltic sprat and Baltic herring contained less fat in the spring and summer. Lipid content of the fish peaked in the autumn. Autumn samples had the lowest water content (63–69%) and spring samples had the highest water content (69–75%). Protein content was quite stabile, in winter on average 15.6 ± 0.4%, in autumn on average 16.2 ± 0.5%, in spring on average 15.9 ± 0.4%, and according to this study no distinct connection between the seasons and protein content of Baltic herring was found.

The autumn, winter-spring and summer batches grouped in different sections in Fig. 3. PLS component 1 explained the skin attribute (more broken) for autumn samples and the shape attribute for winter and spring samples. PLS component 2 described darker flesh color and lower flavor for spring and summer samples. Flesh color was negatively correlated with flavor and also aroma of the Baltic herring (R = −0.46 and −0.47, respectively). Flesh color was darker in spring and summer Baltic herring, because of the spawning season.

Samples from autumn and winter had a higher lipid content (5.9–9.7%) and thus were also sweeter, more flavorful, aromatic and also harder. The lipid content of Baltic herring correlated moderately with flavor, aroma, and greasiness (R = 0.36, 0.39, and 0.46, respectively). No off-flavors were present in autumn and winter caught Baltic herring. Aroma and flavor intensity, or the fishiness factor, was more distinguishable for the summer caught Baltic herring.

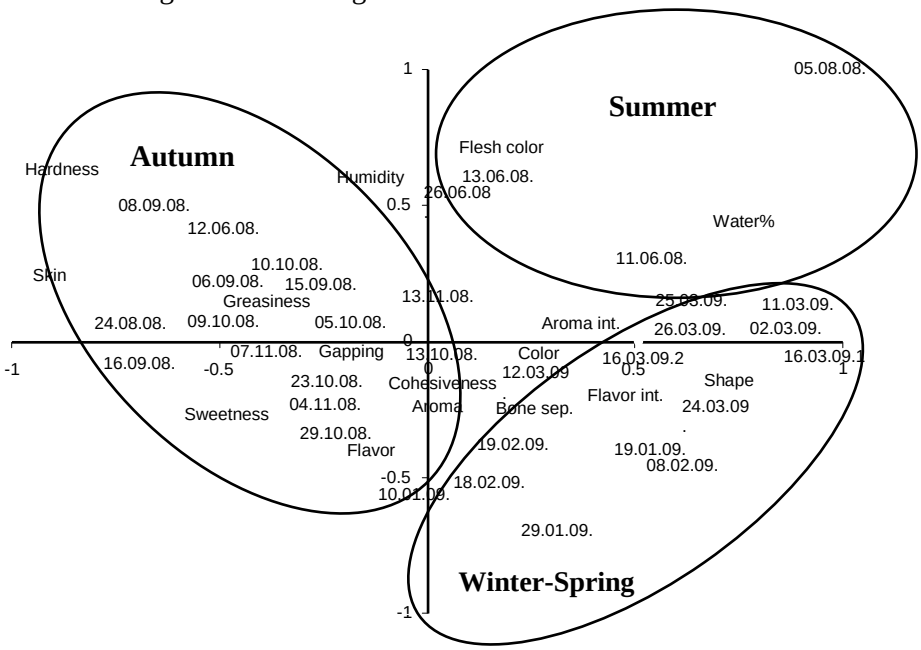

Figure 3. PLS of Baltic herring sensory attributes, herring batches, and water content (batch code DD.MM.YY); x-explained 27% (water%), 20% (sensory attributes); y-explained 31% (water%), 12% (sensory attributes). The circles represent three seasonal groups.

CONCLUSIONS

Composition parameters of Baltic sprat and Baltic herring varied in between catching seasons. Variation in fish water and lipid content during catching season was perceived by the sensory panel. Baltic sprat was described according to sensory and composition measurements better than Baltic herring, probably due to differences in catching techniques. Baltic sprat and Baltic herring caught in the autumn had the highest sensory quality and the highest lipid content. As Estonian fish catching quota is smaller than the fishing power, we recommend that most of the Baltic sprat and Baltic herring should be caught in the autumn. The following work will study the influence of seasonal variation in fish products.

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PUBLICATION II

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Spice-cured sprats ripening and sensory properties development

Journal of Aquatic Food Product Technology, accepted 6 May, 2012

Spice-cured Sprats Ripening and Sensory Properties Development

Journal of Aquatic Food Product Technology

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Abstract

Spice-cured sprat products from fresh and frozen-thawed fish in glass and plastic package and from two different catching years were prepared. Sensory attributes of the spice-cured sprats correlated well with free amino acids content and storage and loss modules. The present study indicates that spice-cured sprats made from frozen-thawed fish and/or packed into plastic package ripen faster and their sensory properties were different in hardness, sourness and rancidness.

Keywords: baltic sprat, ripening, rheology, sensory

Introduction

Baltic sprat (*Sprattus sprattus balticus*) is a small fish species from the Baltic Sea, and products from this fish have been eaten in Baltic and in Eastern European countries for centuries. Due to its small size, sprat and sprat products have been commodity products. Earlier studies by Timberg et al., 2011 have shown high lipid content (12-23%) with richness in essential n-3 fatty acids (30-35 g/100g of total fatty acids) of Baltic sprat which ensures very good nutritional composition. Sprat quota in the Baltic Sea is 225 thousand tonnes for year 2012, which is 22% less than last year and future quotas are also predicted to decrease (European Comission, Fisheries, 2012). Hence it is vital importance to produce sprat products with added value. Traditional Baltic sprat products are spice-cured sprats. Spice-cured sprats are produced mostly in the Baltic countries and the annual volume is about 35 thousand tonnes, which is 40% of the whole Baltic countries sprat catch. Spice-cured sprats are made from whole fish, which have been layered into jars with spices and covered with salt brine. Spice-cured sprats will obtain its characteristic sensory properties during ripening, and they are marketed starting from two weeks of ripening. The end of the shelf-life is from two to three months set by producer based on experience. Traditionally spice-cured sprats were made only from autumn-winter caught fish, which was immediately packed into metal tins. In the industry, for easier processing and all-year round availability, spice-cured sprats are made from all season catches, from fresh and frozen fish, and packed into various packages (metal, glass, plastic). Biological variations between fish stocks, and large variation in processing conditions produce products with unpredictable qualities (Timberg et al.,

submitted 2012), which limits the possibilites of entering new markets. The effect of freezing-thawing depends upon a raw material and processing technology used. Besteiro et al. (1997) found that freezing-thawing of anchovies appears to favour the ripening process. Steffánsson et al. (2000) found also that freezed-thawed herring ripened in a similar manner to fresh salted herring, but faster. In other studies, freezing-thawing has had a negative effect on fish quality (Saeed and Howell, 2004; Kristinsson et al., 2009; Rehbein and Oehlenschläger, 2009; Szymczak, 2011). Package material also influences the product quality and stability, by affecting the environmental conditions. Glass package of spice-cured sprats is very similar to metal package, since both are 100% gas (oxygen) barriers and thus no oxygen is present. In plastic package gas exchange with atmosphere through material or sealing affect the product quality and stability. The influence of freezing-thawing of sprats as well as of package material on the ripening of spice-cured sprats, is however not established.

Most of the research on understanding the ripening/curing process has been done with herring, *Clupea harengus* (Nielsen, 1995; Nielsen and Børresen, 1997;Andersen et al., 2007; Christensen et al., 2010). Herring is also salted as whole fish, but has much larger size (average herring weight about 200 grams compared to sprat 10-15 grams). Also anchovies (*Engraulis*), and sardine (*Sardina pilchardus*) have been studied (Nunes et al., 1997, Mendes et al., 1998). Earlier studies on fish ripening have been done with products which shelf-life is from 4-13 months; however the shelf-life of spice-cured sprats is 2.5 months. Therefore there is a clear need to describe the ripening process of spice-cured sprats to detect what limits the shelf life and influences the product quality. Furthermore, to the authors knowledge, no complex studies have been done on spice-cured sprat ripening to examine free amino acids (FAA) formation, texture and sensory profile development.

Spice-cured sprats can be organoleptically characterized by cured-meat-like flavour, a characteristic sprat odour with the touch of spices, and flesh which is firm and resistant to finger pressure, and which separates easily from backbone. All of these sensory attributes can be related to instrumental measurements. Common instruments for determination of texture in foods are the texture profile analyser (TPA) and rheometer. However there is no one universal method for analysing texture of fish, because of the complex structure, composition and rheology of fish muscle (Barroso, 1998, Badii and Howell, 2002). With very small fish, such as sprat, it is impossible to cut sample pieces from fillets with definite fibre orientation, dimensions, and due to the variation of each individual fish the fluctuation of texture

measurements is very big. Thus, a small deformation rheological method, described by Badii and Howell (2002) was chosen to evaluate the textural properties of spice-cured sprats.

Structural changes during ripening of salted fish take place because of proteolysis (Nielsen and Børresen, 1997; Engvang and Nielsen, 2000). Also effect of protein oxidation has been suggested (Andersen et al., 2007, Christensen et al., 2010). Protein degradation during ripening has been shown to lead to characteristic organoleptic properties of salted fish. The total amount of free amino acids increases during ripening of fish; Kiesvaara (1975) found approximately 6-7 fold increase in the total content of free amino acids during 4-6 month cold storage of spice-salted herring. Amino acid profiles are suitable for monitoring sprat ripening and characterizing the possible differences in processing of fresh and frozen-thawed fish, and effect of glass and plastic package.

Autumn sprats were chosen for the study as the fat content as well as sensory properties are the highest quality (Timberg et al., 2011). The fish used in this study had the characteristic fatty acid profile with a high level of polyunsaturated fatty acids (PUFAs), which are prone to oxidation. Andersen et al., 2007 found that fatty acids composition did not change during the ripening time of salted herring (400 days), and the lipids were only modestly oxidized, with peroxide value from 0.4-1.1 meq peroxide/kg. However for achieving a complete nutritional picture of fatty acids in spice-cured sprats ripening it was decided that one batch of sprats are monitored in fatty acid profile and no special attention to lipid oxidation was attended.

The aim of this study was to describe the process of spice-cured sprats maturation by combining instrumental and sensory methods, and to investigate ripening and stability of the product in different packages and the effect of freezing and thawing of fish before preservation. Microbiological consortia development and changes have also been conducted, but these results will be presented in a different communication.

Materials and Methods

Samples

50 kg of Baltic sprat was obtained from local fisherman in December of 2009 and in November of 2010. Half of the fish was spice-cured at the next day after catching, and half of the fish was frozen. Fish for freezing was packed into zip-lock plastic bags (weight 3.5 kg, dimensions 45x35x3 cm) and was frozen at -40°C for 24 hours, after which fish was

transferred into storage freezer at temperature -18°C. Spice-curing was carried out with a traditional spice mixture (1.7 g / 100g of fish), containing several different spices such as vanilla, cinnamon, cardamom, coriander, ginger, nutmeg, nutmeg flower, allspice, clove, black pepper, and bay leaves. Whole sprats (including heads and guts) were layered into 185 ml glass and plastic jars (O_2 barrier for plastic 1550 ml/m^2/24h, CO_2 barrier 8800 ml/m^2/24h, N_2 barrier 640 ml/m^2/24h) together with the spice-mix, and covered removing air with brine (saturated NaCl solution) sealed with metal or plastic cap. The ratio of fish and brine in the jars was 70:30. Preserved sprats were cured at 4°C and analyzed from two to ten weeks. Frozen sprats were kept at -18°C for three months, after which they were thawed at 4°C during 24 hours, and preserved in glass and plastic jars as preserves from the fresh fish. These procedures correspond to industrial spice-cured sprat recipe and technologies.

Composition Analysis

Lipid, protein, water content, and pH of the samples were measured in triplicate and the results were averaged. All samples (ca 300 g of fish) were filleted, and drained off excess fluids and minced. Water content of the minced fillets was measured using a halogen analyzer (HR 83, Mettler Toledo, Switzerland), the protein content by the Kjeldahl method (Velp Scientifica UDK 142, Italy) For pH measurements the minced fish was diluted with distilled water (1:10) and homogenized, and measured with 744 pH Meter, Metrohm, Switzerland. Samples for the lipid analysis were freeze-dried and measured by the Soxhlet method (Velp Scientifica SER 148 Solvent Extractor, Italy). All necessary reagents were purchased from Sigma-Aldrich, Germany.

Amino acid and free amino acid analysis

For amino acid analysis the homogenized samples were frozen at -40 °C and freeze-dried (Heto PowerDry PL3000, HSC500, Denmark). Free amino acids content was measured after dilution of 0.2 g freeze-dried sample with 20 ml of Milli-Q water and filtration. Total amino acids content was analyzed after acid hydrolysis of freeze-dried powder in 6 M HCl for 24 h at 105 °C in glass tubes sealed under nitrogen. The filtrated samples of both analysis were directly submitted to derivatization procedure utilizing Waters AccQ•Fluor^{TM} Reagent Kit. Chromatographical analysis was carried using an ACQUITY UPLC system (Waters, USA), equipped with a C18 column (BEH C18, 100×2.1 mm, 1.7 µm, Waters, USA) and a photo

diode array (PDA) detector ACQUITY PDA 2996 (260 nm). Waters AccQ•Tag Eluent A and Eluent B were used as mobile phase of analysis. All analyses were conducted in triplicate for each sample. Amino acid standards used for external calibration were obtained from Serva (Germany). Waters Empower 2 chromatography software package was used for data acquisition and management.

Fatty acid analysis

The fatty acid profile of sprat samples fillets was determined after derivation of lipids into methyl esters (FAMEs) according to the standard EVS-EN ISO 5509:2000. Bligh&Dyer (1959) method was used for lipid extraction. The methyl esters samples were injected to the gas chromatograph (Agilent 7890A GC System) equipped with the flame ionization detector (FID) at a split ratio of 1:10. Helium served as the carrier gas (flow 1 ml/min). Agilent J&W GC Column HP-88 (60 m x 0.25 mm x 0.2 µm) was used for the separation of FAMEs. The analytical conditions were: injector port temperature -250°C and detector temperature -280°C. The oven was programmed from 125°C to 230°C (125°C, hold time 1 min; 125 °C to 145 °C, ramp 8 °C/min, hold time 10 min; 145 °C to 190 °C, ramp 5 °C/min; 190 °C to 230 °C, ramp 1,5 °C/min). Retention times and intensities of the external standard (Supelco 37 Component FAME mix, Sigma-Aldrich, Germany) were used to quantify chromatographic peaks of the sample.

Texture

Small deformation rheological analysis was measured according to Badii and Howell (2002). Minced fish paste was prepared by mixing 90 g minced fish with 10ml distilled water and homogenized at 11000 rpm for 2 min (Polytron PT 2100, Kinematica, Switzerland). The sample was measured with a rheometer (Physica MCR301, Anton Paar, Austria) using a temperature sweep from 25 to 90°C and cooling back to 25°C at a heating rate 2 °C/min. The stress was 1 Pa and frequency 1 rad/sec. A 40 mm parallel plate geometry and a gap of 2 mm was used.

Descriptive Sensory Analysis

A trained panel of 6 panellists was used for descriptive sensory analysis to describe the spice-cured sprat products. All of the panellists had previous experience in descriptive sensory

analysis with various food products including at least 50 h evaluation of seasoned sprats and were employees of the Competence Centre of Food and Fermentation Technologies in Tallinn, Estonia. The panellists were trained further for this test during five sessions each lasting 1.5 hours, where the attributes, definitions and reference materials were agreed upon. During these sessions the panellists had access to commercial samples similar to the samples to be tested. Samples were described by their appearance, flavour and texture attributes, described in detail by Timberg et al., 2012 (submitted). All of the dried seasonings were measured in the amount of 5 ml individually into medium-sized sniffing glasses, covered with watch glasses. Two attributes for appearance were used: shininess and meat colour. Shininess was defined as degree of skin shine from dull to shiny. Meat colour was defined as meat colour intensity from light to dark. Seventeen attributes for flavour were used: overall spiciness, allspice, nutmeg, nutmeg flower, cinnamon, bay leaves, black pepper, vanilla, clove, ginger, coriander, cardamom, fish, sweet, sour, salty, and rancid. Spices intensity was defined for each individual spice as their flavour intensity. Fish was defined as fish flavour intensity. Three attributes for texture were used: hardness, moistness, and greasiness. Hardness was defined as the strength that is needed to cut completely through fish meat with molars. Moistness was defined as the amount of moisture released while pressing the fish meat against palate with tongue. Greasiness was defined as amount of grease left on mouth surfaces.

The samples were evaluated in triplicate. The sensory laboratory was equipped with individual booths and computers according to ISO 8589-2007. The panellists used a data collection program, written internally, to enter scores. A scale with 0.5 point increments, where 0 = none and 15 = very strong, was used. Unsalted crackers and purified filtered water was available at all times, as well as reference materials and definition sheets. The panellists were told to clean their palates in between the samples. The samples were served on white ceramic plates, coded in three-digit numbers. Each panellist was served two whole fish for evaluation. The serving of the samples was randomized. The samples were prepared 30 minutes and references 30 minutes to two hours ahead of testing. The samples were stored at 2-6 °C before evaluation.

Data analysis

A 2^3 factorial design was used in the present study. The three variables, or factors, which were used in the study were: freezing-thawing, package, and batch. XL Stat version 2011.1.04 (XL Stat, New York, NY, USA) was used for data analysis. Analysis of Variance (ANOVA) was performed and significant differences (p=0.05) among samples were found using Fisher's protected LSD for fatty acid and amino acid analysis results. Pearson correlation coefficients were calculated between instrumental and sensory measurements. Descriptive data was mapped with free fatty acid and glutamate and glycine content data using Principal Component Analysis (PCA). Mathworks Matlab R2007b with Statistics toolbox was used for t-test (p=0.05) analysis of nutritional composition and pH analysis data.

Results

Changes in chemical composition during ripening

The chemical composition of cured sprat fillets was monitored in two different batches of sprats, which were caught in autumn 2009 and 2010. The average lipid content of the sprats was 9.4%±1.1 and 12.1%±1.7, for batch 2009 and 2010 respectively, and the level did not change significantly during the 10 weeks of ripening (Tab.1). The water (66.0%±0.7 and 66.9±1.4 respectively) content in the fish fillet was also relatively stable throughout 10 weeks; no statistically significant differences during the ripening and storage were observed. The protein content in the fish fillet (13.8±0.6 and 14.9±0.33 for 2009 and 2010 respectively) decreased throughout the entire ripening period about 30% (Tab.1). The decrease in protein content can be explained by protein hydrolysis during ripening. Like protein content the pH in the fish muscle as well as in brine slightly decreased throughout the entire ripening period (Fig.1). The pH in the brine was lower than in the fish, which might be due to the difference in buffering capacity. There was a tendency that brine pH was slightly lower in glass jars than in plastic jars and that brine pH was slightly lower in samples made from frozen-thawed fish than in samples made from fresh fish (Fig.1). The phenomenon was observed for batches of both years 2009 and 2010. The salt content of the sprats and brine equilibrated within the first four weeks, and there was no difference between fresh and frozen-thawed fish. Thereafter, the salt concentration remained constant for the rest of the ripening period, being 6% in fish. Earlier studies have shown, that freezing-thawing can increase salt penetration

during brining of herring (Deng, 1977). However this was not observed in this study, probably because the size of the sprat is much smaller.

Also the decrease in pH can be explained by protein hydrolysis, namely by the decrease of the ratio between basic and acidic amino acids (B/A) (Kiesvaara, 1975), which was observed in case of all samples (Tab.2, Fig.2b). According to the sensory analysis the samples were considered ripened when the ratio B/A was around 1.0. Similar value for anchovy was suggested by Hernández-Herrero et al., 1999.

During ripening of sprat the FAA concentration increases in fish muscle, data is shown for batch 2010 (Fig.2a). The total FAA content of samples made from frozen-thawed fish increased approximately 4 fold during 2.5 month ripening for both packages glass and plastic. In samples made from fresh fish the total of FAA increased more in plastic package (6.4 fold) than in glass package (4.6 fold). With confidence interval of 85% total FAA content increased more in samples made from fresh fish than in samples made from frozen and thawed fish. The highest amounts of FAAs were detected in samples made from fresh fish and packed into plastic package, which showed the fastest rate of ripening. Thus it can be concluded that spice-cured sprats in plastic package ripen faster, regardless of pre-treatment of fish and samples made from frozen-thawed fish ripened faster than samples made from fresh fish. That was also in good agreement with pH measurement.

After ten weeks, in all samples the most abundant amino acids were glutamic acid, leucine and lysine (Tab.2), such as observed earlier in ripened herring (Kiesvaara, 1975) and in ripened sardine (Nunes et al., 1997). Total degree of FAA formation from fish protein reaches the value of 30%, which is in good accordance with loss of protein in cured sprats (Tab.1). Free histidine concentration was 143±4 mg/100g and 203±7mg/100g, in fresh and frozen-thawed fish respectively. While other FAA concentrations started to increase in proportion with total FAA concentration, then histidine concentration in samples acted curvilinearly (Fig.2c). It decreased in the beginning of the ripening, and from the fourth week histidine concentration started to increase. During the process of ripening samples made from frozen fish started to contain more histidine than those made from fresh fish.

Fatty acid composition

The sprats had the characteristic fatty acid profile with a high level of PUFAs (Tab.3). The first four abundant fatty acids (in decreasing order) were oleic (c18:1), palmitic (c16:0), docosahexanoic or DHA (c22:6n-3) and eicosapentaenoic or EPA (c20:5n-3) acids. PUFAs

represented the most dominant class of fatty acids (37.8 % of total fatty acids), followed by mono-unsaturated fatty acids or MUFAs and saturated fatty acids or SFAs with 32.0% and 30.0% of total fatty acids, respectively. From poly-unsaturated fatty acids (PUFAs) n-3 was present more than n-6, exhibiting a n-3/n-6 ratio of 8.32±1.1 on average. In general the sprat fatty acid composition was stable during processing, only slight increase in MUFA 18:1 and decrease of total PUFAs was observed in frozen-thawed ripened sprats. The effect can be explained by prooxidants such as metals and myglobin and hemoglobin in sprat blood (Kanner et al., 1988; Apte and Morrisey, 1987) and enzymes released during freezing-thawing cycle which are responsible for fatty acid metabolism (Richards and Li, 2004).

Texture

Viscoelastic measurements of spice-cured sprats muscle homogenate are presented for the samples ripened for 10 weeks made from fresh fish in plastic jars (Fig.3a) and in glass jars (Fig.3b). In all cases, prior to heating, the storage modules (G') was found to be greater than the loss modules (G''), indicating that the homogenate had a gel-like structure (Fig.3, Tab.4). Values of G' and G'' after heating from 25 to 90°C are shown in Table 4. Cooling from 90 to 25°C resulted in a noticeable increase in both the storage (G' 25) and loss (G'' 25) moduli due to hydrophobic interactions (Howell and Lawrie, 1985). The storage modules (G') and loss modules (G'') values varied among different catching years (Tab.4), which can be explained by biological variability of fish.

The storage modules (G') and loss modules (G'') values of ripened sprats were higher in samples in glass jars than in plastic jars, for both samples made from fresh and frozen-thawed fish (Tab.4). This is in accordance with hydrolysis data and indicates that samples ripened at a faster rate in plastic package. There is a tendency that samples made from frozen-thawed sprats have higher storage modules (G') and loss modules (G'') values at 8 weeks of shelf-life, than samples made from fresh sprats. However, this was noted only for batch 2010. This finding supports the theory that frozen-thawed samples do not always acquire similar texture properties as samples made from fresh fish. Total FAA content correlated with storage modules (G') and loss modules (G'') values being at 25°C, -0.64 for both moduli.

Sensory analysis

Sensory attributes differentiated spice-cured sprat samples according to ripening time (Fig.4a). Principal component one (PC1) was described by fishiness and hardness on the one

end and moistness and general spiciness on the other end. PC2 was described by total FAA and Glu+Gln content on the one end and salty taste on the other end. General spiciness and moistness of samples had a tendency to rise with ripening time, this was noted for all samples, regardless of catch, pre-treatment and package. Hardness of samples correlated well with ripening time, less ripened samples were harder and more ripened samples were softer. This can be explained by hydrolysis of the protein content during ripening which caused the softening of the fish muscle. Hardness also correlated positively with storage modules (G') and loss modules (G'') values measured at 25°C, 0.64 and 0.57, respectively. Hardness had a negative correlation with total FAA content (0.73), rancidness (0.68) and sourness (0.72). Spice-cured sprat samples packed into plastic jars had lower hardness than spice-cured sprats in glass jars. Fish flavour intensity of samples had a tendency to be lower in more ripened samples. Fish flavour could be associated with FAAs formation during ripening (correlation -0.47). No variation in different spices was noticed. PC3 differentiated samples according to rancid flavour on the one end, and shine and colour on the other end (Fig.4b). Total FAA content correlated positively with rancidness (0.44) and moistness (0.73). Spice-cured sprat samples made from frozen-thawed fish had a tendency to be more sour, and rancid taste developed more rapidly than samples made from fresh fish. Samples made from fresh fish were shinier and had a characteristic colour. Spice-cured sprat samples in plastic jars also had a tendency to have higher sourness and rancid flavour.

Discussion

The aim of this study was to reveal to which extent catch, pre-treatment and package influence the ripening process of spice-cured sprats by combining sensory and instrumental analysis.

A catch of wild fish will have a large biological variation between individual fish and this eventuates in large standard deviations in analysis results. For example Refsgaard et al., (1998) found that in farmed salmon the biological variation could contribute by up to 20% of the observed variation in the data obtained from individuals from the same batch and same family, this can only be higher in wild fish such as sprat. The variation in fish quality between catches in different seasons and years or even between different catches in same year can be even bigger. This was clearly demonstrated also in this study where rheological as well as sensory properties of cured sprats of catches autumn 2009 and autumn 2010 were different.

Total content of FAA increased during the ripening process of spice-cured sprats. Similar increase of total FAA content during fish ripening has been reported by several authors (Kiesvaara, 1975, Nielsen and Børresen, 1997, Olsen and Skara, 1997, Mendes et al., 1998, Nunes et al., 1997). There was a tendency that spice-cured sprat samples made from fresh fish and samples packed in plastic package the total of FAA increased more, which indicates faster rate of ripening. Faster ripening in plastic containers than in glass containers with similar geometry can be explained by permeability of plastic package to gases, especially to oxygen which can affect the microbial processes as well as modify proteins by formation of sulphide bridges in proteins.

B/A ratio of spice-cured sprats showed that optimum quality of samples was achieved upon sixth week of ripening. Sample made from fresh fish and packed into glass maintained this stable quality until the end of ripening experiment. The B/A ratio of other samples continued decreasing and thus the optimum quality was lost. Spice-cured sprats in plastic package and from frozen-thawed fish ripen faster.

High histidine concentration of sprats was expected as it is known that clupeid fish (Telostea, Clupeoidei) such as sardines, anchovies and herring have relatively high histidine levels in the muscle (Love, 1980; Yamanaka et al., 1986). Free histidine is decarboxylated by bacterial enzymes into histamine (Rehbein and Oehlenschläger, 2009; Nollet and Toldrá, 2010) and thus the concentration of histidine in fish decreases. After four week of ripening the proteolysis increased and this liberated more FAAs from muscle protein, and the histidine content starts to rise in the same manner as other FAAs. Mendes et al., (1998) found that the the loss of histidine is proportional to histamine production and decrease of histidine was noted during 20 days of ripening of fresh and frozen sardine. Similar changes in spice-cured sprat samples were likely to take place. Freezing-thawing influenced histidine content of spice-cured sprats, being higher in the beginning and in the end of ripening process. This was also noted in the ripening experiment with sardines (Mendes et al., 1988). Higher histidine content in frozen-thawed fish can probably be explained as freezing and thawing as additional production phases will destruct fish muscle and leaching FAAs to the brine is more extensive.

Direct sprat fillet texture analysis could not be applied. Optimal texture analysis method which takes into consideration test condition parameters such as sample geometry, sample location and probe or testing device geometry is easier to design with bigger fish species (Veland and Torrisen, 1999). Texture analysis of smaller fish e.g. herring (Schubring and

Oehlenschläger, 1997; Stefansson et al., 2000; Christensen et al., 2010) is more difficult because fillets are smaller and there is limited material for choosing most suitable part of the fish muscle with definite dimensions. Thus small deformation rheological analysis was used as only option for very small fish species, e.g. sprat. Texture of homogenized spice-cured sprat samples had a gel-like structure. This finding is also in line with results from earlier studies by Saeed and Howell (2004) of mackerel homogenate rheological behaviour. After slight increase in G' and G'', when heated from 25 to 40°C a larger increase followed at around 45°C, which can be ascribed to denaturising the light meromyosin (tail) (LMM) and heavy meromyosin (HMM) chains (Sano et al., 1988; Sano et al., 1989). In addition to HMM (Sano et al., 1988; Sano et al., 1989), other proteins also contribute to the G' increase, including water-soluble sarcoplasmic proteins and actin, which denaturate at 48-52 and 65°C, respectively (Hastings et al., 1985; Jensen and Jorgensen, 2003). Texture analysis indicated that spice-cured sprats in glass jars had harder texture than sprats in plastic jars, for both samples made from fresh and frozen-thawed fish. The storage modules (G') and loss modules (G'') values were higher in spice-cured sprats in glass jars than in plastic jars, both samples made from fresh and frozen-thawed fish, which indicated that samples ripened at a faster rate in plastic package. That was in good agreement with rate of protein hydrolysis. Texture analysis also showed the variation in samples made from frozen-thawed fish. This shows that spice-cured sprats made from frozen-thawed raw material do not always acquire the same texture as same product made from fresh sprats, which makes the product quality unpredictable. Even though small deformation rheological analysis gave good correlations with sensory attributes and with the rate of protein hydrolysis, the deviations between parallels and batches were higher than expected. This might suggest that suitability of small deformation rheological analysis in spice-cured sprats ripening will need further study.

From sensory attributes general spiciness and moistness of samples had a tendency to be higher in more ripened samples, in all variations (fresh and frozen, glass and plastic). This was in good agreement with texture analysis, where more ripened samples had lower G' and G''. In the beginning of ripening the total content of FAAs is low and fishiness is thus very well perceived. The disappearance of fishiness during ripening can be related to FAA increase, and especially to glutamate and glutamine, which provide the sample an umami taste will mask fishiness flavour. More ripened samples had tendency to have higher general spiciness and individual spices, but significant difference was not observed. This might be explained by spice-mixture, which distributed randomly among sprats in the jars, and the

actual spiciness of the fish meat could not be distinguished from the sensations caused by spices. Spice-cured sprat samples packed into plastic jars had lower hardness, and higher sourness and rancidness than spice-cured sprats in glass jars. This is in good agreement with rheological measurements of spice-cured sprats as well as pH. Sourness could be the effect of higher content of acidic FAAs, and rancidness is a result of oxidation processes where more MUFAs and PUFAs degraded in the frozen-thawed samples during ripening. Spice-cured samples made from frozen-thawed fish had a tendency to be harder, and sour and rancid taste developed more rapidly than samples made from fresh fish. Freezing and thawing will damage the protein native structure and makes them susceptible to further reactions (Badii and Howell, 2002) so deterioration process is likely to begin and develop faster in these samples. Sensory analysis results show that within different catching years the ripening pattern is similar and differences are mainly due to the nutritional composition (lipid content).

Conclusions

The present study indicates that spice-cured sprats made from frozen-thawed fish and/or packed into plastic package ripen faster according to both sensory and instrumental analysis and their texture and flavour properties are different. Chemical components which correlate well with the sensory attributes of the spice-cured sprats are free amino acids and storage and loss modules. Those findings can be utilized by the sprat processing industry in product quality and stability control.

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Appendices

Table 1. Water, lipid and protein content (%) of spice-cured sprats made from fresh and frozen-thawed fish in glass and in plastic package.

Sample	Composition	Ripening time					
	%	Day 0	Week 2	Week 4	Week 6	Week 8	Week 10
Fresh glass (2009)	water	64.4±1.4	65.6±1.3	66.8±0.8	66.5±0.9	67.9±1.1	68.6±0.8
	lipid	10.9±1.3	10.8±0.7	8.8±0.9	9.3±1.0	8.9±0.9	9.3±0.9
	protein	13.4±0.2	11.3±0.1	11.5±0.1	10.7±0.0	10.7±0.9	9.9±0.5
Fresh plastic (2009)	water	64.4±1.4	65.5±0.9	66.8±1.1	68.6±0.9	67.9±0.7	67.6±1.2
	lipid	10.9±1.3	10.7±0.8	10.1±1.3	10.0±1.1	9.3±0.8	9.6±0.9
	protein	13.4±0.2	12.6±0.9	11.7±0.2	11.8±0.3	11.3±0.7	10.8±0.5
Frozen glass (2009)	water	68.1±0.6	67.1±1.0	66.9±0.7	67.6±0.6	68.6±0.4	68.5±0.6
	lipid	11.9±0.9	8.9±0.3	9.6±0.1	8.9±0.2	8.5±0.9	9.8±0.7
	protein	14.5±0.6	11.6±0.3	10.2±0.1	10.9±0.2	10.6±0.9	9.9±0.3
Frozen plastic (2009)	water	68.1±0.6	69.2±1.0	70.9±1.2	68.8±1.3	70.7±1.0	69.9±0.4
	lipid	11.9±0.9	8.2±0.3	8.8±0.2	9.2±0.2	9.0±0.7	9.9±0.5
	protein	13.5±0.6	11.3±0.2	10.9±0.2	10.8±0.3	9.8±0.3	10.3±0.1
Fresh glass (2010)	water	67.3±1.0	62.0±0.2	66.6±1.0	68.1±0.7	67.4±1.5	68.3±0.4
	lipid	13.9±1.2	12.9±0.8	10.6±0.1	10.8±0.3	10.1±1.5	9.9±0.3
	protein	15.1±0.4	11.8±0.5	11.3±0.2	10.5±0.3	10.1±0.2	9.9±0.7
Fresh plastic (2010)	water	67.3±0.9	67.0±0.1	68.5±0.9	62.5±0.8	60.4±0.8	62.9±1.5
	lipid	13.9±1.2	15.2±0.2	10.1±0.7	11.8±0.2	11.8±0.3	11.6±0.8
	protein	15.1±0.4	12.9±0.7	11.6±0.2	12.1±0.3	11.3±0.2	11.1±0.1
Frozen glass (2010)	water	65.6±1.7	65.0±1.3	65.0±1.1	68.5±1.4	68.6±0.9	60.3±0.1
	lipid	11.1±1.4	11.9±0.7	9.7±0.5	9.5±0.6	8.3±1.8	11.3±0.6
	protein	14.8±0.1	14.8±0.4	11.9±0.5	11.4±0.4	11.4±0.6	10.1±0.0
Frozen plastic (2010)	water	65.6±1.7	61.6±1.3	64.3±1.3	60.6±1.8	61.3±1.8	61.2±1.3
	lipid	11.1±1.4	13.5±1.6	10.4±0.5	13.2±0.5	13.8±0.4	12.7±0.5
	Protein	14.8±0.1	11.4±0.4	12.1±0.5	11.1±0.4	10.4±0.6	10.1±0.0

Values are expressed as means ± SD

Table 2. Effect of freezing and ripening on amino acid (AA) and free amino acid (FAA) composition (mg/100g of fish) of fresh, frozen-thawed, and ripened Baltic sprats in glass and in plastic jars. The result are given for year 2010.

Amino acid	Fish		Frozen-thawed	Fresh glass 10weeks	Fresh plastic 10weeks	Frozen glass 10weeks	Frozen plastic 10weeks
	AA	FAA	FAA	FAA	FAA	FAA	FAA
Ala	955±46	65±6 d	70±1 d	138±8 c	250±26 a	165±12 b	174±18 b
Arg	1075±47	15±1 c	23±13 c	186±7 a	140±12 c	91±10 b	95±10 b
Asn	43±38	1±1 b,c	1±1 c	2±1 b,c	20±4 a	1±0 b	3±0 b,c
Asp	1535±10	6±1 d	22±1 c	139±9 b	202±22 a	201±15 a	213±17 a
Cys	30±5	3±0 c	1±0 c	7±6 b	8±1 a,b	11±1 a	8±1 a,b
Gln	ND	13±1 e	9±1 e	240±12 c	400±281 d	188±19 b	143±15 a
Glu	2092±11	19±2 e	41±1 d	108±9 c	175±19 b	199±14 a	206±17 a
Gly	715±103	25±1 d	25±1 d	63±2 c	128±7 a	67±6 c	75±9 b
His	546±5	143±3 c	203±7 a	122±4 d	159±7 b,c	206±39 a	178±14 b
Ile	616±40	9±6 d	16±0 d	104±4 c	157±13 a	113±9 b,c	115±14 b
Leu	1111±58	24±2 d	31±0 d	196±8 c	279±22 a	222±17 b	227±27 b
Lys	1606±17	34±5 d	53±1 d	193±17 c	306±45 a	241±16 b	236±26 b
Met	416±20	12±1 d	13±0 d	101±4 c	135±7 a	108±9 b,c	114±14 b
Phe	568±13	13±0 d	15±0 d	128±4 c	180±4 a	127±10 c	149±15 b
Pro	515±48	11±1 d	15±0 d	71±3 c	108±9 a	81±6 b	88±11 b
Ser	638±33	17±1 d	32±0 c	96±4 b	93±7 b	112±12 a	108±11 a
Thr	686±28	17±1 d	20±0 d	86±4 c	131±10 a	93±7 b	97±10 b
Tyr	493±28	13±0 d	15±0 d	113±4 b	147±3 a	110±9 b	99±10 c
Val	727±41	19±2 d	25±0 d	120±5 c	201±16 a	134±10 b	139±16 b
Total	14527±4	457±22 d	632±12 d	2214±106 c	3221±248 a	2470±224 b	2468±253 b
B/A	0.59	2.78 a	2.13 b	1.00 c	0.68 e	0.78 d	0.71 d,e

Values are expressed as means ± SD; means with the same letter within a row were not significantly different at p<0.05 level; ND-not detected. B/A ratio Basic (Lys, His, Arg)/Acidic (Asp, Thr, Ser, Pro, Glu)

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Table 3. Effect of freezing and ripening on fatty acid composition (% of total fatty acids) of fresh, frozen-thawed, and ripened Baltic sprats in glass and in plastic jars. The result are given for year 2010.

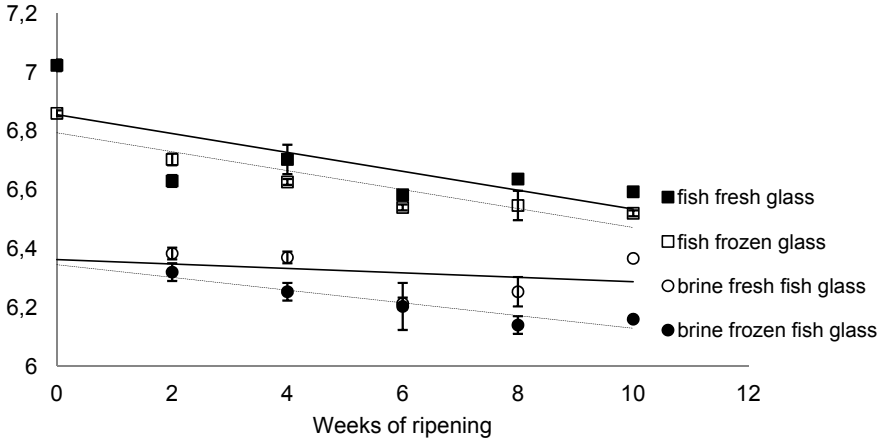
Fatty acid	Fresh	Frozen-thawed	Fresh glass 10w	Frozen glass 10w	Fresh plastic 10w	Frozen plastic 10w
14:0	4.98±0.26 a	4.61±0.08 b	4.78±0.02 a,b	4.73±0.13 a,b	4.93±0.23 a	4.83±0.16 a,b
16:0	22.08±0.52 b	22.54±0.40 a,b	23.11±0.17 a	23.12±0.62 a	23.23±0.18 a	22.53±0.61 a,b
18:0	2.25±0.03 b	2.42±0.12 a,b	2.46±0.01 a,b	2.69±0.58 a	2.63±0.02 a,b	2.25±0.03 b
∑ SFA	29.30±0.73 c	29.58±0.35 b,c	30.35±0.19 a,b,c	30.54±1.13 a,b	30.80±0.38 a	29.61±0.68 b,c
14:1	0.26±0.01 a	0.22±0.00 a	0.17±0.14 a	ND	0.24±0.01 a	ND
16:1	5.89±0.16 a	5.29±0.05 b	5.78±0.07 a	5.26±0.39 b	5.41±0.12 b	5.26±0.13 b
18:1	24.46±0.83 b,c	25.29±1.15 b	23.97±0.12 c	28.87±0.62 a	24.90±0.31 b,c	27.82±0.46 a
20:1	0.48±0.03 a	0.36±0.12 a,b	0.20±0.17 b,c	0.06±0.11 c,d	0.34±0.04 a,b	ND
22:1	0.67±0.02 a	0.65±0.02 a,b	ND	ND	ND	ND
∑ MUFA	31.77±0.77 b	31.81±1.23 b	30.12±0.27 c	34.20±0.30 a	30.89±0.33 b,c	33.08±0.38 a
18:2n6	3.13±0.06 a,b	2.89±0.18 b	3.07±0.07 a,b	3.27±0.12 a	3.10±0.13 a,b	3.05±0.30 a,b
18:3n6	0.11±0.00 a,b	0.10±0.00 b	0.10±0.00 b	ND	0.10±0.01 b	ND
18:3n3	3.12±0.07 a,b	3.10±0.03 b	3.06±0.03 b	3.30±0.22 a	3.01±0.09 b	3.19±0.04 a,b
20:2n6	0.53±0.01 a	0.51±0.02 a	0.48±0.01 a	0.13±0.22 b	0.51±0.01 a	ND
20:3n3	0.27±0.01 a,b	0.26±0.01 b	ND	ND	ND	ND
20:5n3	9.05±0.03 a	8.78±0.19 a,b,c	8.93±0.09 a,b	7.95±0.31 d	8.74±0.08 b,c	8.60±0.09 c
22:2n6	0.60±0.00 a	0.59±0.01 a,b,c	0.60±0.01 a,b	0.53±0.02 d	0.58±0.01 b,c	0.57±0.01 c
22:6n3	22.07±0.51 a	22.37±1.19 a	23.29±0.35 a	20.08±1.14 b	22.23±0.68 a	21.90±0.77 a
∑ PUFA	38.88±0.53 a,b	38.58±1.59 a,b	39.52±0.49 a	35.26±1.33 c	38.28±0.69 a,b	37.31±0.97 b

Values are expressed as means ± SD; means with the same letter within a row were not significantly different at p<0.05 level; ND-not detected.

Table 4. Storage (G') and loss (G'') modulus values obtained at 90°C (G' 90, G'' 90) and on cooling to 25°C (G' 25, G'' 25) for spice cured sprats samples made from fresh and frozen-thawed fish, stored in glass and in plastic jars for 2, 8 or 10 weeks. Values are means with standard deviation.

Sample	Ripening time	G'_{90} (Pa)	G'_{25} (Pa)	G''_{90} (Pa)	G''_{25} (Pa)
Fresh glass (2009)	2 weeks	2667±85	13567±850	306±45	2737±32
	8 weeks	2720±150	13400±520	425±93	2950±312
	10 weeks	2778±169	11687±2347	425±82	2693±194
Fresh glass (2010)	2 weeks	2383±296	19933±2483	330±27	4003±497
	8 weeks	1450±92	8440±198	240±35	1585±49
	10 weeks	1230±6	7350±14	193±11	1830±17
Fresh plastic (2009)	2 weeks	2615±191	13250±919	363±72	2650±269
	8 weeks	1823±155	9505±455	260±35	1935±231
	10 weeks	1550±58	1755±1630	96±17	1463±43
Fresh plastic (2010)	2 weeks	2345±148	11000±283	270±11	2240±226
	8 weeks	1135±133	6213±527	162±19	1450±173
	10 weeks	657±42	3615±799	101±7	851±182
Frozen glass (2009)	2 weeks	1903±136	11415±1434	316±25	2443±306
	8 weeks	2720±155	12800±1273	425±93	2793±405
	10 weeks	2862±339	14025±1401	451±69	3348±515
Frozen glass (2010)	2 weeks	2705±304	13750±1202	337±88	3130±368
	8 weeks	3230±236	17000±424	435±21	3680±28
	10 weeks	1617±78	8590±692	258±10	1830±111
Frozen plastic (2009)	2 weeks	1290±45	8150±845	202±13	1943±305
	8 weeks	1897±55	9587±520	275±20	1887±257
	10 weeks	1458±119	9880±170	227±36	2615±134
Frozen plastic (2010)	2 weeks	2145±7	11850±636	243±23	2445±134
	8 weeks	2325±35	11300±1414	351±11	2440±212
	10 weeks	720±42	3570±354	196±7	840±99

Fig.1. pH of Spice-cured sprats and brine in glass and in plastic package made from fresh and frozen-thawed fish. Number 0 is fresh fish and numbers 2-10 are showing the week of ripening. The results are given for year 2010.



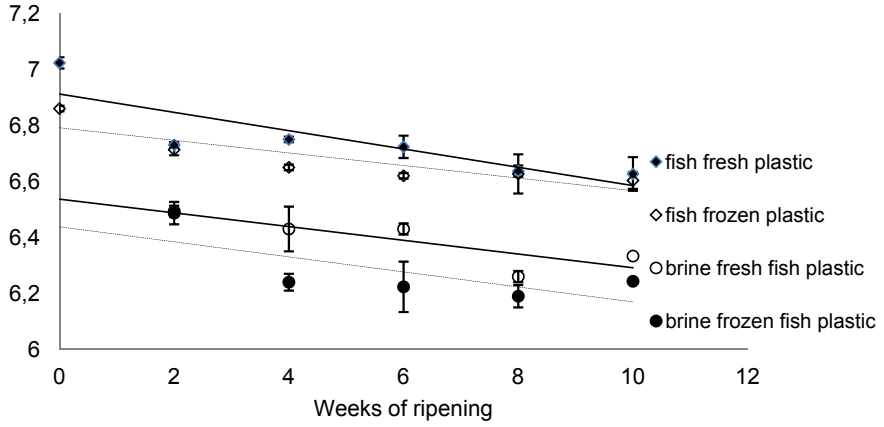
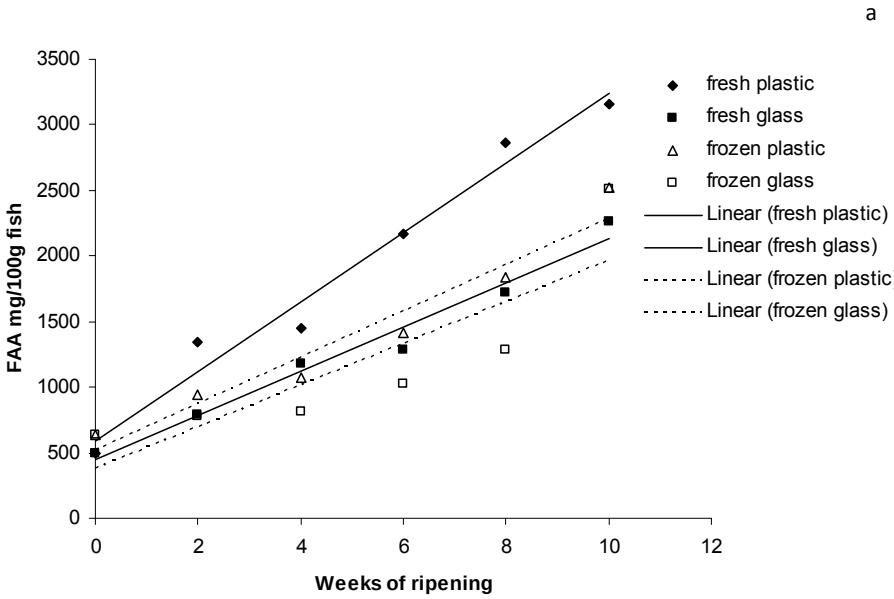
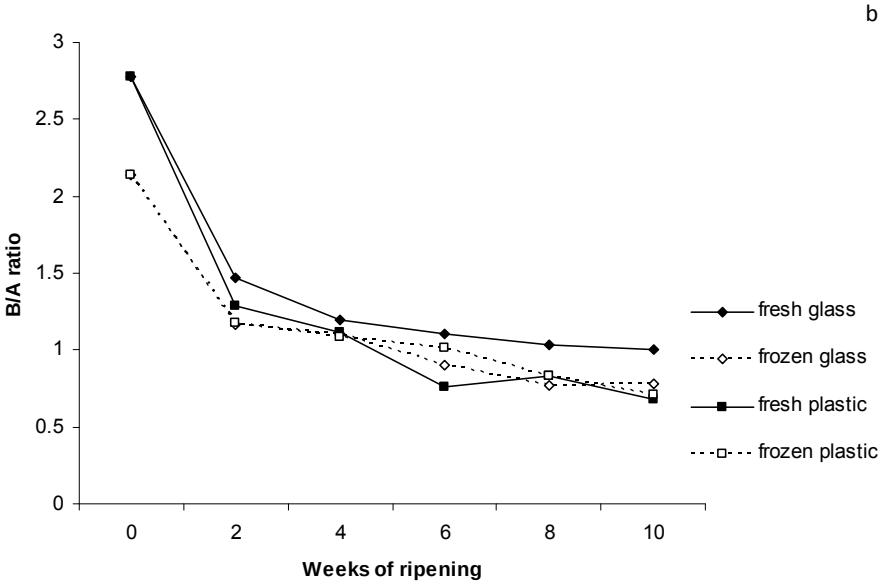


Fig.2. FAA content (a), Basic and acidic (B/A) amino acids ratio (b), and histidine (His) content (c) of spice-cured sprats in glass and in plastic package made from fresh and frozen-thawed fish. Number 0 is fresh fish and numbers 2-10 are showing the week of ripening. The results are given for year 2010.





c

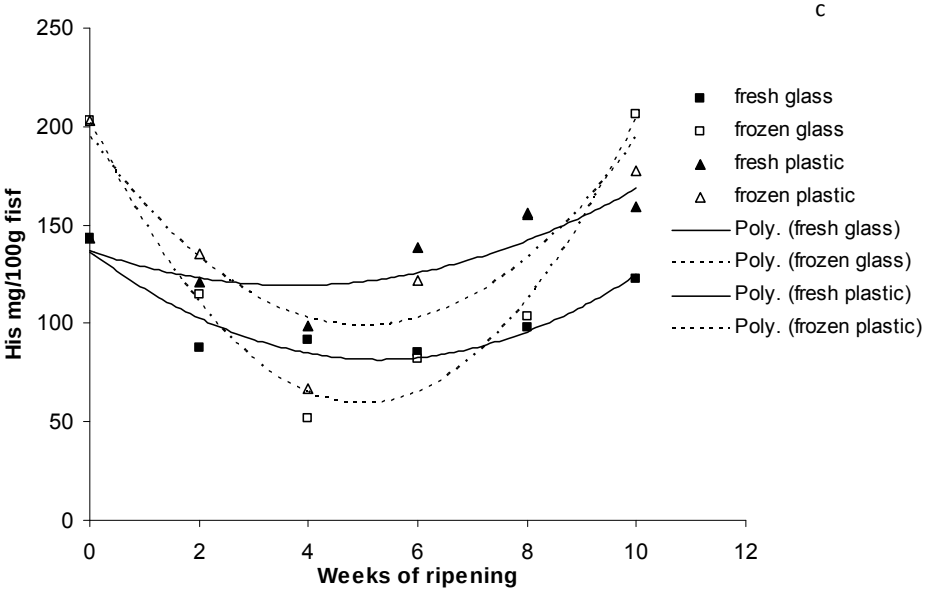
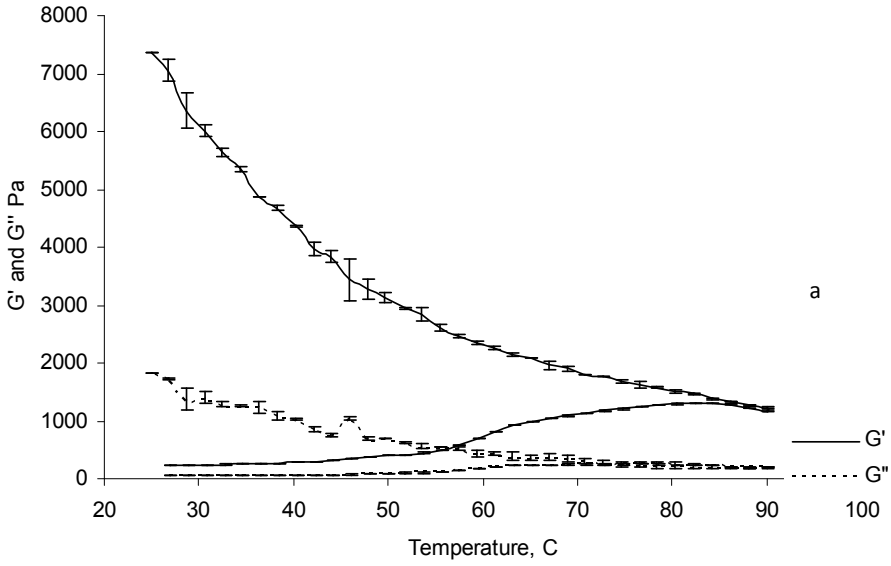

250
200
150
100
50
0
His mg/100g fisf
0
2
4
6
8
10
12
Weeks of ripening
fresh glass
frozen glass
fresh plastic
frozen plastic
Poly. (fresh glass)
Poly. (frozen glass)
Poly. (fresh plastic)
Poly. (frozen plastic)

Fig. 3. Temperature sweep from 25 to 90C and from 90 to 25C for homogenised spice-cured sprats made from fresh fish and ripened for 10 weeks (a) glass jars and (b) 10 plastic jars. The result are given for year 2010.



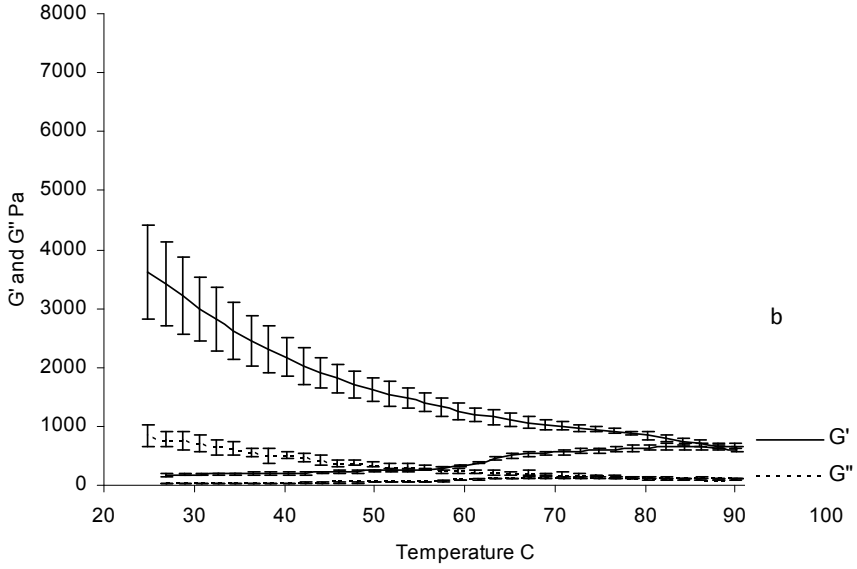
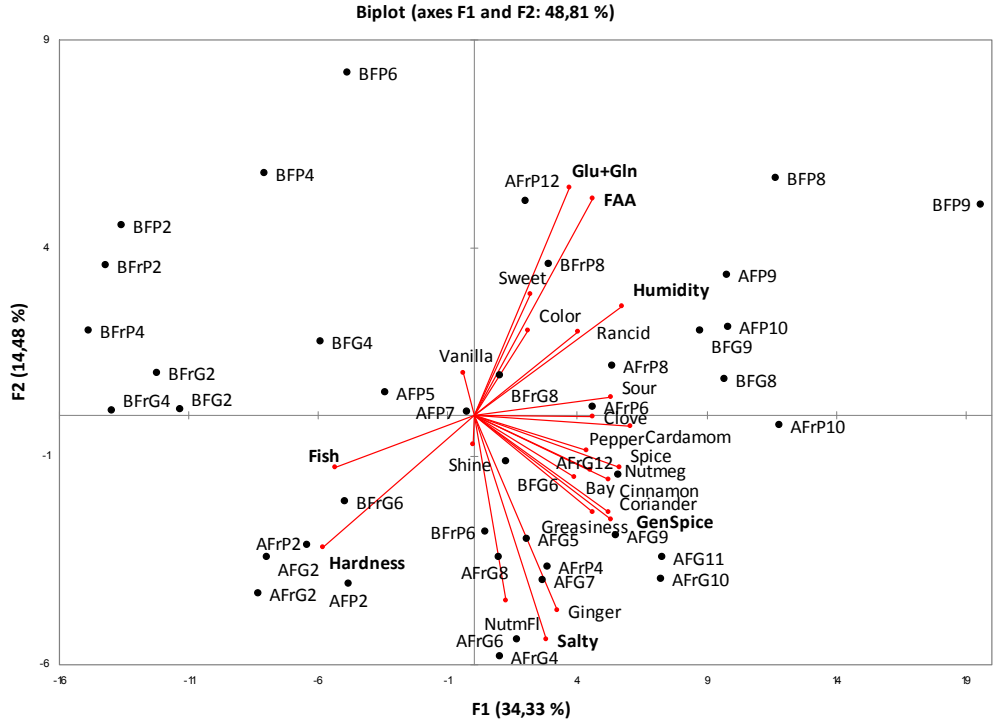
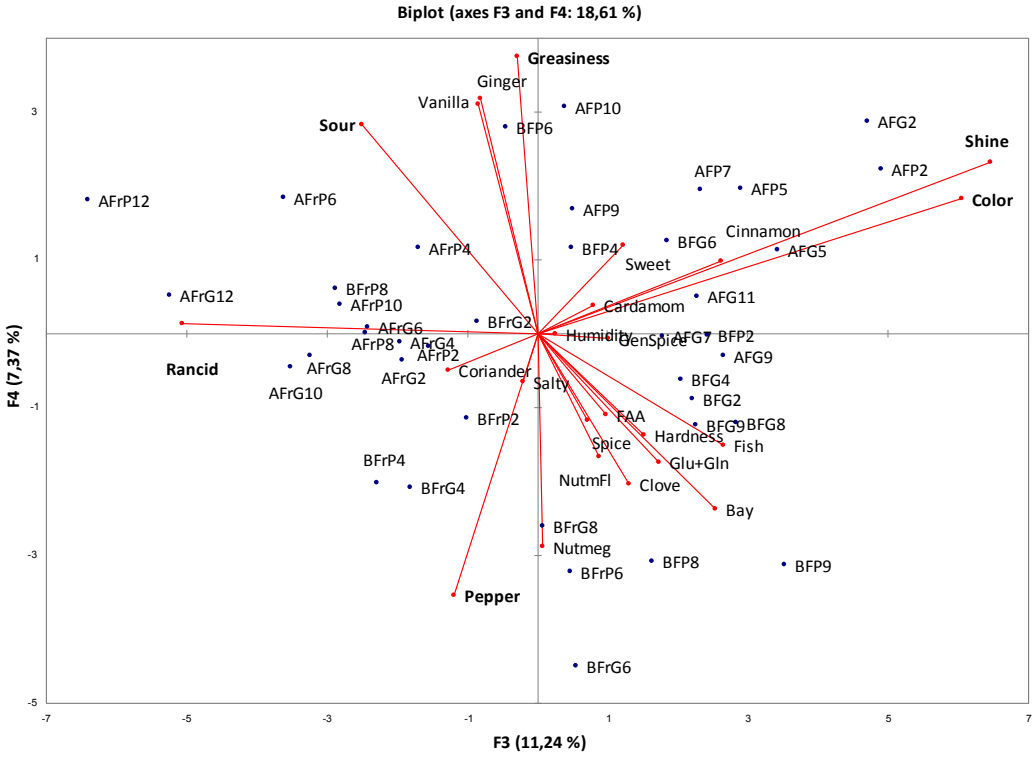


Fig. 4. Sensory attributes, total free amino acid (FAA) and glutamic acid+glutamine content (Glu+Gln) on PCA of spice-cured sprat samples made from fresh (F) and frozen (Fr) fish in plastic (P) and in glass (G) package, year 2009 (A) and 2010 (B). Numbers 2-12 are showing the ripening week.




Biplot (axes F3 and F4: 18,61 %)
Greasiness
Ginger
Vanilla
AFP10
Sour
BFP6
AFG2
Shine
AFP7
AFP2
AFP5
AFrP12
AFrP6
AFP9
Color
Cinnamon
AFrP4
BFP4
BFG6
AFG5
Sweet
AFrG12
BFrP8
AFrP10
AFG11
Cardamom
BFrG2
AFrG6
Humidity
GenSpice
AFG7
BFP2
AFrP8
AFrG4
AFrP2
AFG9
Rancid
AFrG8
Coriander
Salty
BFG4
AFrG2
AFrG10
BFG2
BFrP2
FAA
BFG9
BFG8
Hardness
Fish
Spice
BFrP4
Glu+Gln
NutmFl
Clove
BFrG4
Bay
BFrG8
Nutmeg
BFrP6
BFP8
BFP9
Pepper
BFrG6
F4 (7,37 %)
F3 (11,24 %)
3
1
-1
-3
-5
-7
-5
-3
-1
1
3
5
7

PUBLICATION III

Timberg L, Koppel K, Kuldjärv R, Chambers IV E, Soontrunnarudrungsri A, Suwonsichon S, Paalme T

Seasoned sprat products acceptance in Estonia and in Thailand

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Seasoned Sprat Products Acceptance in Estonia and in Thailand

Journal of Aquatic Food Product Technology

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Keywords: acceptance, baltic sprat, clustering, consumer, cured, flavor

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Abstract

The aim of the study was to describe and evaluate competitiveness of Estonian spice-cured sprat products in Estonia and in Thailand. Sample A was saltiest, sample B had lowest overall spiciness, and sample C had highest pepper flavor and sour taste intensity. The main drivers of consumer acceptance for the spice-cured sprat products were different, flavor and appearance in Estonia and appearance and odor in Thailand. In Estonia one cluster of consumers liked the traditional (sample A) and lightly spiced (sample B) sprat products, while the other cluster liked the traditional (sample A) and the marinated (sample C) sprat products. In Thailand all samples scored low, but manual clustering indicated that marinated (sample C) sprat products are most acceptable. The current study showed that spice-cured sprat products in general can be accepted by Thai consumers, especially as part of meals, if further flavor development is carried out with the products.

Keywords: acceptance, baltic sprat, clustering, consumer, cured, flavor

Introduction

A variety of fermented and cured fish products are consumed all over the world. Probably the most familiar products are fish sauces, which are primarily used for seasoning different dishes. There are also a number of cured or fermented products consisting of whole fish, such as Swedish Surströmming, Mediterranean Anchovy, Korean Hongeohoe and Japanese Kusaya, which are usually consumed without further preparation as an appetizer or a side dish (Tamang and Kailasapathy, 2010). Spice-cured sprats are very similar to cured anchovy products, with a difference in the seasoning mixture and curing extension.

Spice-cured sprat products have been produced in Eastern-Europe for centuries, and thus can be referred to as a traditional product or regional product. However, in Estonia these products are not registered as Protected Designation of Origin (PDO, Eur-Lex EC 510/2006). Cured and fermented fish products are also well known in Asian countries such as Thailand (Steinkraus, 2004; Paludan-Müller et al., 2002), which makes sprat products a potential export article from Eastern European countries.

Spice-cured sprats are prepared from whole fish, including heads and intestines. Sprats (*Sprattus sprattus balticus*) are layered into tins or jars together with the spice-mix, and covered with salt brine. Spice-mix contains several different spices such as allspice, clove, black pepper, coriander, nutmeg, nutmeg flower, cinnamon, cardamom, ginger, vanilla and bay leaves. Sprats are cured at 4 °C at least for two weeks before they are considered ready for consumption. Traditionally spice-cured sprats have been prepared only from sprats caught in the autumn, because during that time sprats contain the most fat (up to 23%, Timberg et al., 2011), and this gives the product its distinctive succulent texture and flavor. Currently spice-cured sprats are

prepared all year round and a range of spice-cured sprat products have evolved from the traditional product, varying in the degree of curing, spiciness, and saltiness.

There is an increased interest in traditional food at the retail and consumer levels, which has been observed by several researchers, e.g. Vanhonacker et al., (2010). Increasing globalization of the food sector enables traditional products to emerge into new markets (e.g. Verbeke and Lopez 2005; Hersleth et al., 2011). The major part of the production of traditional food products is manufactured by small- and medium-sized enterprises (SMEs) (Vanhonacker et al., 2010). SMEs usually cannot afford large-scale marketing campaigns for product launch in new markets, thus it is important to study consumer acceptance and attitudes in the target market.

Consumer response to new food products is related to previous experience with similar types of products (Bredahl 2003; Verbeke et al., 2010), or in the case of first time exposure, positive associations with already familiar attributes are crucial. New products can be expected to be accepted in the markets where consumers have previous experiences with characteristic attributes of product in question (Hersleth et al., 2011). For this reason consumers with different familiarity of products from two countries differing in local cuisine and spice-cured fish products consumption - Estonia and Thailand - were included in this study. However, it is known that consumers often vary in product liking and tastes (Cleaver and Wedel, 2001; Schilling and Coggins 2006). The heterogeneity of consumer preferences and the importance of identifying consumer segments with different preference patterns demands in-depth data analysis with suitable clustering methods.

Computerized statistical package clustering (CSPC), especially agglomerative hierarchical clustering (AHC) has been found useful in consumer preference testing (Schilling and Coggins 2006). AHC is a statistical technique that is utilized to group numbers together based on patterns

for a certain response (Ward 1963; Everitt et al., 2001). However, CSPC may result in clusters with a large number of consumers who might not like/dislike the same products as other people in that cluster (Yenket et al., 2011). CSPC is found on the modeling of cluster means rather than individual respondents, and different clustering algorithms can give very different solutions and relationships with the sensory data, and there is no clear way of deciding which to choose (Cleaver and Wedel, 2001). Thus the correctness of one computerized statistical package cannot be tested with another computerized statistical package, but it can be assessed with manual clustering (MC) methods. MC methods enable analysis of how well consumers fit within the statistical clusters. Yenket et al., (2011) demonstrated combination of CSPC and MC objectivity with two separate consumer studies. Thus, in the current study consumer acceptance clusters of spice-cured sprat products were first modeled with AHC, and their fitting was tested with MC methods.

The objective of this study was to determine whether consumers in Estonia and Thailand accept similar flavor combinations in spice-cured fish products or whether the traditional Estonian products would need to be modified for export. In addition, drivers of overall liking for traditional and new product were predicted.

Materials and Methods

Samples

Three samples A, B, and C (Table 1) were studied. Sample A represented the traditional preparation of spice-cured sprats, where whole sprats together with brine and seasoning are packaged in tin cans. Sample B consisted of lightly spiced vacuum-packaged seasoned sprat

fillets and sample C was seasoned and marinated sprat fillets in oil, which were packaged in glass jars. All of the samples were packed in the current production facilities of a local fish products manufacturer. The samples tested in Estonia and in Thailand were from the same batch, and studies were done within a period of one month. The samples to be tested in Thailand were packaged and shipped using express mail service. The size of the spice-cured sprats in sample products was in range of 10-15 grams. The samples were stored at 2-6 °C before evaluation. The spice mixture was the same for all three samples, and varied only in amount. Three spice-cured sprat samples with distinctive sensory attributes and currently available combinations of seasoning level, product appearance, marination, packaging type and medium were studied because the intent was to compare products that matched currently available ones in Estonia.

Descriptive Sensory Analysis

A trained panel of 6 panelists used descriptive sensory analysis to describe the seasoned sprat products. Five panelists were female, and one panelist was male, in the age range of 21 to 31 years. All of the panelists had previous experience in descriptive sensory analysis with various food products including at least 50 h evaluation of seasoned sprats and were employees of the Competence Center of Food and Fermentation Technologies in Tallinn, Estonia. The panelists were trained further for this test during five sessions each lasting 1.5 hours, where the attributes, definitions and reference materials were agreed upon (Table 2). During these sessions the panelists had access to all the samples to be tested. Samples were described by their appearance, flavor and texture attributes. Similar orientation for sensory testing was used by Koppel et al., (2011), Drake and Drake (2011) and Elia (2011).

The samples were evaluated in triplicate. The sensory laboratory was equipped with individual booths and computers according to ISO 8589-2007. The temperature of the evaluation room was 21-23°C. The panelists used a data collection program, written internally, to enter scores. A scale with 0.5 point increments, where 0 = none and 15 = very strong, was used. Unsalted crackers and purified filtered water was available at all times, as well as reference materials and definition sheets. The panelists were told to clean their palates in between the samples. The samples were served on white ceramic plates, coded in three-digit numbers. These procedures are similar to those used by Koppel and Chambers (2010) and Koppel et al., (2011). Each panelist was served two fillets (10-15 grams) for evaluation; sample A was gutted and filleted before serving. The serving of the samples was randomized. The samples were prepared 30 minutes prior to testing and references were prepared 30 minutes to two hours prior to testing, and covered with plastic food wrap. The samples were stored at 2-6 °C before evaluation.

Consumer Study

The central location trials were conducted in Bangkok, Thailand, and in Tallinn, Estonia. 106 consumers in Thailand and 111 consumers in Estonia (Table 3) were recruited based on willingness to try cured fish products. The consumers were asked to fill in a questionnaire with questions about overall liking, appearance, aroma, texture, flavor, fish flavor, salty taste, and aftertaste liking on a 9 - point scale where 1 – dislike extremely and 9 – like extremely. Intensity of texture, flavor, fish flavor, salty taste, and aftertaste was evaluated on a 9-point scale where scores 1 - 4 were summed as “too low”, 5 was “Just About Right”, and 6 - 9 as “too high” intensity. Purchase intent was evaluated on a 5 - point scale where 1 – “I definitely would not buy this product“ and 5 – “I definitely would buy this product”. A check-all-that-apply (CATA)

question was used to determine consumer attitudes towards the samples. In both countries the CATA included: I would eat this product; I would eat this product as a snack; this product would be used as part of a meal in my home; it has a pleasant flavor; it has a familiar flavor. In Thailand additional questions included: similar with canned fish product in Thailand; different from canned fish product in Thailand; I would eat this product with rice; I would add this product to instant noodles. In Estonia the CATA question included options like: I would eat this product with bread; I would eat this product with potatoes.

At the end of testing consumers completed a demographic ballot with questions about gender, age, education level, fish products consumption frequency, fish products purchase locations, and the type of products that were consumed at least once per month.

The consumers were served two fillets (10-15 grams) of each sample - in a randomized order on a disposable plate, coded with three-digit numbers. The samples were prepared 60 minutes prior to testing. The samples were stored at 2-6 °C before evaluation. The consumers were encouraged to take a bite of an unsalted cracker and drink some purified water between the samples.

Composition Analysis

Lipid, protein, dry weight, and salt contents of the samples were measured in triplicate and the results were averaged. Sample A was gutted and all samples drained of excess fluids before further analysis. All samples were homogenized using a homogenizer (Polytron PT 2100, Kinematica, Switzerland) at speed of 11000 rpm. Water content of the samples was measured using a halogen analyzer (HR 83, Mettler Toledo, Switzerland). The protein content of the fish samples was measured by the Kjeldhal method (Velp Scientifica UDK 142, Italy). The lipid

content of fish was measured by the Soxhlet method (Velp Scientifica SER 148 Solvent Extractor, Italy). The salt content of samples was measured according to AOAC Official Method 937.07. All necessary reagents were purchased from Sigma-Aldrich, Germany.

Data analysis

Consumer data was analyzed using XL Stat version 2011.1.04 (XL Stat, New York, NY, USA). Analysis of Variance (ANOVA) was performed and significant differences (p=0.05) among samples were found using Fisher's protected LSD. The consumers were clustered according to overall liking using Agglomerative Hierarchical Clustering (AHC) and by manual clustering (MC) methods: Strict, Strict Liking Only, Loose and Loose Liking Only, described by Yenket et al., (2011). AHC (Ward 1963; Everitt et al., 2001) was preformed using Ward's method. A dissimilarity plot and a dendrogram were utilized to determine how many clusters were appropriate for analysis. Dissimilarity among panelists within two clusters was small and the dendrogram also confirmed that two clusters for this study are optimum, because the sample size was small (n=3) and breaking the data into more than two clusters caused several groups to have fewer than five consumers. MC procedures for selecting consumers for each manual cluster based on most frequently liked products, or most frequently liked and most frequently disliked products. Strict MC consisted of consumers who gave the most frequently liked product and the most frequently disliked product their highest and lowest scores, respectively. Strict Liking Only MC consisted of consumers who gave the most frequently liked product their highest score. Loose MC consisted of consumers who gave the most frequently liked product and the most frequently disliked product either their highest or next to highest score and lowest or next to lowest scores, respectively. Loose Liking Only MC consisted of consumers who gave the most

frequently liked product their highest or next to highest score. The larger (size) cluster was named EST1/THAI1 and smaller cluster was named EST2/THAI2. Consumer clusters overall liking mean scores were mapped using Principal Component Analysis (PCA) with descriptive sensory analysis data added as supplemental variables. AHC and MC methods were compared based on most frequently liked/disliked products hedonic versus rank scores.

Results

Nutritional composition

All samples had comparable protein content from 11.8% to 13.5% (Table 4). Sample C had higher lipid content (16.3%) because it was packed in vegetable oil. There was some variation in salt content of the samples. Sample A had the highest salt content because this product was packaged in saturated salt brine. Sample B and C had lower salt content, because these samples were salted before packaging and there was no salt in packaging media. Water content of the samples A and B was similar, 61.2 % and 63.8% respectively. Sample C water content was slightly lower, 57.2%, probably because the product was immersed in vegetable oil.

Descriptive Sensory Analysis Data

Fish, overall spice, black pepper, bay leaf, spice, nutmeg, and clove flavors and salty, sweet, and sour tastes were present in all samples (Table 5). The samples were not different for allspice, nutmeg, bay leaf, black pepper, vanilla, clove, coriander, or cardamom flavor intensities; in fact, most of these flavors were barely noticeable. Ginger flavor was not detected in any of the samples. There were no differences between samples in moistness and greasiness perception,

perhaps because the panelists associated moisture and grease as both providing lubrication and did not differentiate between those two attributes.

Sample A had darker meat color than samples B and C, probably because sample A was made from whole fish with intestines and thus was more cured. Sample B was lower in overall spiciness and the flavor of nutmeg flower, cinnamon and cardamom were not detected. Sample C was distinguished as highest in sour taste, and lowest in fish flavor. Higher sour taste was expected because sample C was lightly marinated and spice-cured while other samples were only spice-cured. Sample C, which was seasoned more heavily with the traditional spice mix, had higher pepper flavor intensity ($p>0.05$).

The sensory profile of spice-cured samples A, B, and C was further used to visualize how the samples and consumer clusters differ by the results of the principal component analysis (Fig.1.).

Consumer data

Mean scores and JAR-scores

In Estonia sample A received highest liking scores for almost all attributes, apart from the salty taste (Table 6). Sample A scored the most "Just About Right" answers in flavor (42%), fish flavor (55%), and texture (66%) attributes. Sample B was liked less than sample A, but more than sample C. Sample B scored the most "Just About Right" answers for salty taste (46%) and aftertaste (59%) attributes. Sample C was rated lowest for all evaluated attributes. Sample C received the least "Just About Right" scores in almost all attributes, with the exception of texture, where the score was similar to sample B score.

In Thailand sample C received the highest scores in all liking attributes. Sample C scored the most "Just About Right" answers in flavor (33%), fish flavor (41%), and aftertaste (40%) attributes. Samples A and B both received similar low mean scores for liking attributes, ranging from 3.7 to 5.3, and there was no significant difference between the two samples in most of the attributes. The exception was salty taste, where sample A was significantly lower in liking (3.7) than sample B (4.4) and C (4.6). Sample A received the fewest "Just About Right" scores for almost in all attributes, except in texture intensity where sample A got the most "Just about Right" scores (32%).

Consumers gave similar scores to 'flavor' and 'fish flavor' in both countries, which indicates 'fish flavor' is the main driver in the flavor of spice-cured sprats (correlation coefficient 0.754). Estonian and Thai consumers had similar attitudes towards the texture of the samples, which they found to be too soft.

Overall liking

Overall Estonians liked sample A and Thai consumers found sample C most acceptable. Regression modeling, with 'overall liking' as the dependent variable, and the other variables as explanatory variables, and a dummy variable on market, gave different interaction variables for Estonia and Thai, 0.681 and 0.599, respectively. This indicates that Estonian consumers acceptance of spice-cured sprat products was higher, proably because spice.cured sprats is a familiar product. Correlation coefficients among 'overall liking' and other variables showed that highest coefficients in Estonia were: flavor (0.711), appearance (0.688), and fish flavor (0.611); and in Thailand: appearance (0.677), odor (0.579), and fish flavor (0.572).

Purchase intensions

After tasting the samples consumers were asked to estimate their purchase intentions. Overall Estonian consumers gave higher scores than Thai consumers. Estonians said that they would like to buy sample A (mean score 3.2). There was no significant difference between samples B (2.5) and C (2.3), purchase intentions. Thai consumers said that they would like to buy sample C (3.0) or sample B (2.8) showing a clear difference in the types of products that would be most successful in the domestic and export market.

Consumer clusters

Agglomerative hierarchical clustering (AHC) according to 'overall liking' results showed that there were two clusters in both countries, in Estonia (EST1, EST2) and in Thailand (THAI1, THAI2). 'Overall liking' was chosen for the clustering driver, because consumers evaluate all sample variables under 'overall liking', and thus it represents best acceptance of a sample. In Estonia AHC cluster EST1 (55% consumers) liked samples A and C while cluster EST2 (45% consumers) liked samples A and B (Table 7). MC method Strict showed that in cluster EST1 25 (42%) consumers liked product A the most and B the least. Because product A and C mean values were not significantly different, this illustrates that consumer in EST1 liked products A and C equally.

Strict Liking Only (SLO), Loose (L), and Loose Liking Only (LLO) affirmed that a large percentage of consumers (52-60%) in cluster EST1 had similar opinions, they liked samples A and C and disliked sample B. The remaining 40% of the EST1 consumers rated all products equally. The highest score was given to sample A by 37%, to sample C by 27%, and equal scores to samples A and C by 15% consumers in cluster EST1. Lowest score was given to

sample B by 50% both consumer clusters EST1 who rated sample A or sample C the highest. Highest score was given to sample B by 34%, to sample A by 32%, and equal scores to samples A and B by 30% consumers in cluster EST2. Lowest score was given to sample C by 76% and 81% consumer clusters EST2 who rated sample B or sample A the highest. Cluster EST2 was more uniform than cluster EST1, and the combining factor was the dislike of product C (68-92% of consumers according to different MC methods).

In Thailand the smaller THAI2 (21% consumers) consumer cluster did not like any of the samples (<5), and the large cluster THAI1 showed all samples were liked slightly (scores of 5.3 to 5.6). The MC method Strict showed that in cluster THAI1 24 (29%) consumers rated product C the highest and A the lowest, but there was no significant difference over all consumers in the cluster among the three samples. Other MC methods (SLO, L, and LLO) affirmed that a large percentage of consumers (46-61%) in cluster THAI1 had similar opinions, they rated sample C the highest and sample A the lowest. According to MC clustering THAI2 was more uniform than cluster THAI1. Different MC methods showed that 59-77% of members THAI2 rated products similarly.

Sensory drivers of liking for clusters

Clustering results combined with descriptive data explained consumer liking of samples further (Fig 1). Sample B had a lot of fish flavor and was harder than other samples. Sample C had lowest fish flavor from all tested samples, and had a sour taste. Differences in fish flavor and sourness liking divided Estonian and Thai consumers into clusters.

Demographics

Education patterns were similar in both Thai clusters; about 30% of members had some high school/high school, and 70% of members had some college/college education (Table 3). In Estonia 70% of cluster EST1 members had some high school/high school and about 20% had some college/college degree. Cluster EST2 members education was mostly college/college education 47% and graduate/professional degree 46%. Estonian consumers with higher education tended to like samples A and B, which had intensive fish flavor, and consumers with less education liked samples A and C, probably because they were similar in overall spiciness. In fish consumption frequency the most marked option was "at least once a week/several times a week" in both countries (Estonia 68% and 76%; Thailand 65% and 68%).

Consumer attitudes

After tasting each sample, consumers were asked to answer a check-all-that-apply (CATA) question: results are shown on Fig 2 and Fig 3. CATA options were different in Estonia and in Thailand, based on local diet character. "It has a pleasant flavor" and "It has a familiar flavor" choice by Estonian consumers was similar, 56% and 52%, 33% and 26%, and 47% and 46%, for samples A, B and C, respectively. These results indicate that consumers may find "familiar flavor" and "pleasant flavor" terms to be a pair or a match. The last two statements aimed to describe with what food item spice-cured sprat products could be consumed. All samples got a little higher agreement for bread than for potato. In all CATA statements sample A was recognized most, as this is the most traditional and familiar product for Estonian consumers. Thai consumers did not think that the products were "Similar with canned fish product in Thailand" (2-7%), and found the products "Different from canned fish products in Thailand"

(64-67%). This result was expected, as these spice-cured sprat products though similar in some ways to Thai fish products, are new to Thai consumers. Thai consumers felt that most familiar and pleasant was sample C. "This product would be used as a part of a meal in my home" was checked by 66%, 66%, and 63%, for samples A, B, and C, respectively. Thai consumers thought that spice-cured sprat products go better with rice (33-42%) than with instant noodles (13-16%).

Discussion

The most characteristic attributes which differentiated spice-cured sprat products and consumers acceptance were saltiness, fishiness and sourness. This was expected as the main difference in products was due to the salt and spice mixture content, and marination. Saltiness intensity sensory scores by sensory panel correlated well with concentration of salt in the products, and indicated that sample C and B had similar and lower saltiness, while sample A was the saltiest product.

Consumer data average scores suggested that in Estonia sample A and in Thailand sample C were the most liked samples, but clustering results explained that different consumer clusters prefer different spice-cured sprat products, and no final decision about product potential on the market cannot be made only according to average liking scores. Spice-cured sprat products scored lower in Thailand than in Estonia. As these spice-cured sprat products are totally new to Thai market the low scores are critical for the producer since this product's experience will probably not ensure repurchase. First time product experience effects on consumer food choice are also in line with the findings of earlier studies by Grunert (2003). In the case of both Estonian and Thai consumers, an increasing percentage of respondents who call the samples

"Just About Right" resulted in an increasing degree of liking. JAR results revealed that Estonian consumers liked their products to have more intense fish flavor, and Thai consumers liked the product with less fish flavor. In Thailand saltiness of spice-cured sprat samples scored very low, indicating that saltiness should definitely be decreased. From correlation coefficients it can be elicited that most likely the main drivers for overall liking in Estonia are flavor and appearance, and in Thailand appearance and odor of spice-cured sprats because the correlation between degree of flavor/appearance and overall degree of liking was the highest.

In Estonia Sample A was highly liked in both clusters, probably because it is an oldest traditional spice-cured sprat product and all Estonian consumers are familiar with the flavor of the product. This also corresponds with results by Guerrero *et al*. (2009) who showed that changes of sensory properties in traditional products are not welcome, and most traditional products are liked best. Researchers expect that most consumers in one cluster would find the same products most and least liked. Consumer clusters according to AHC method may not provide researchers and product developers all the data necessary. For a consumer study it is particularly difficult if all samples presented will get even scores with no statistical difference. This was also the case with cluster THAI1. Conducted manual clustering (MC) showed how well consumers fit under AHC modeled clusters in terms of the most and least liked products. Thus, using manual clustering to supplement the statistical package clustering method provided more information on the members of cluster THAI1, showing a clearer differentiation among samples. The data indicates that sample C would probably be the most accepted product. MC methods demonstrate the actual ratio of most/least liked products in different clusters, which makes it easier to decide the market share and segment of each sample. This is especially relevant when developing and adapting a product for a new market. Statistical clustering and manual clustering methods showed a good

match, and thus it can be concluded that the chosen statistical clustering method suited well for both consumers who are familiar and consumers who are unfamiliar with spice-cured sprat products.

CATA results revealed interesting information, because in acceptance questions, Estonian consumers liked sample B more than sample C, but CATA results showed that consumers may like sample C over sample B. This contradictory circumstance can be explained by consumer clusters. Although sample C average scores were low, there were consumers who liked it very much, and thus they have checked CATA statements. Sample B was a product more neutral in flavor and did not initiate strong liking or disliking in consumers, and thus fewer CATA statements were checked.

Demographics data demonstrated that consumers in larger clusters, both in Estonia and in Thailand, had lower fish consumption frequencies "at least once a week/several times a week" than the other cluster in the same country. This can be explained by the age division of clusters, as older consumers consume fish more frequently (Myrland et al., 2000).

Over 60% of Thai consumers stated that they would use spice-cured sprat products as a part of a meal at home, which would require further research in order to understand how and in which dishes they would like to use these spice-cured sprat products. This could give more detailed information how current spice-cured sprat products should be developed, so they would be purchased by Thai consumers.

Conclusions

Three Eastern European traditional spice-cured sprat products, different in their flavor characteristics, were evaluated by consumer panels in Estonia and in Thailand. Estonian

consumers liked samples with more intense fish flavor, and Thai consumers liked the sample with the sourest taste, and disliked saltiness intensity. Estonians and Thais are both used to fermented and cured fish products, but the flavor preferences are different. Overall liking drivers for spice-cured sprat as a traditional product in Estonia were flavor and appearance, and in new market- Thailand appearance and odor. Albeit Thai consumers mean scores for spice cured sprat products were very low (below 5-neither like or dislike), in the CATA statements over 60% of consumers stated that they would use this product at home as a part of a meal. Statistical clustering and manual clustering methods comparison showed a good match, and thus it can be concluded that the chosen AHC statistical clustering method suited well to this analysis, although the combination of AHC clustering and manual clustering provided additional insight into acceptance patterns. The current study showed that spice-cured sprat products in general can be accepted by Thai consumers, especially as part of meals, if further flavor development is carried out with the products.

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Figures and tables

Figure 1 Estonian and Thai consumer clusters (Estonia 1 and Estonia 2, and Thai 1 and Thai 2, respectively) flavor liking scores PCA, descriptive sensory data added as supplementary variables

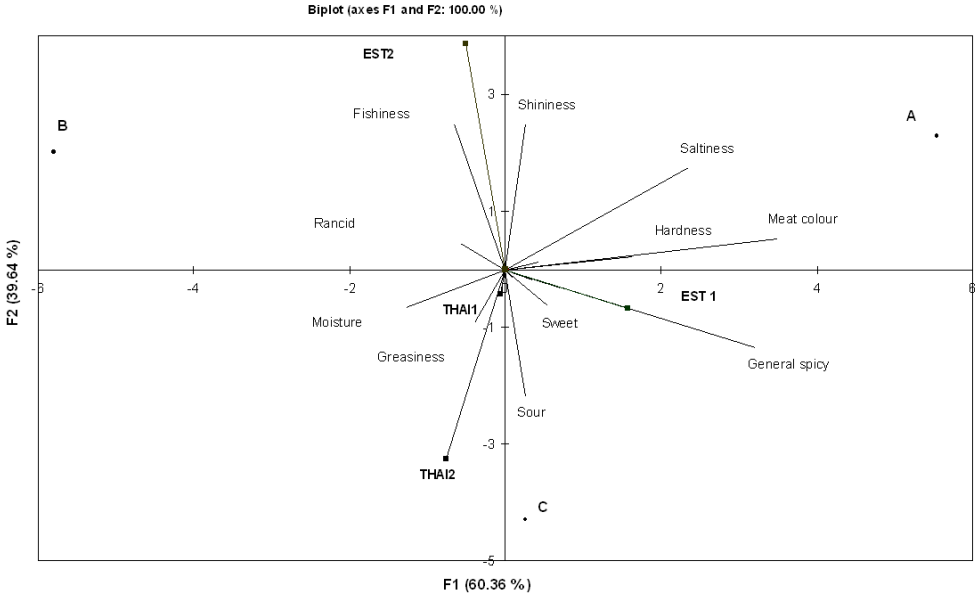

Figure 2 Estonian consumer check-all-that-apply results

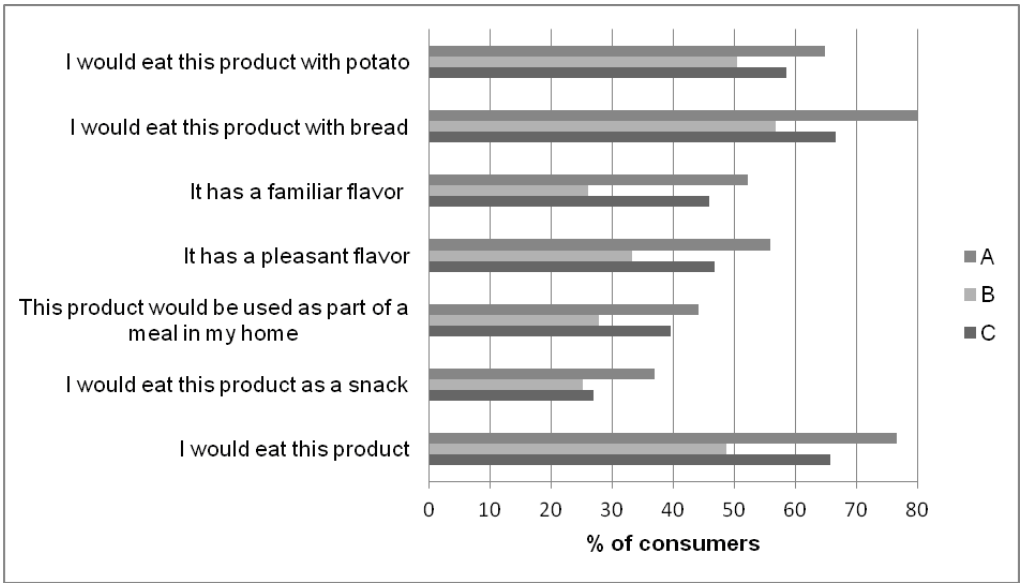

Figure 3 Thai consumer check-all-that-apply results

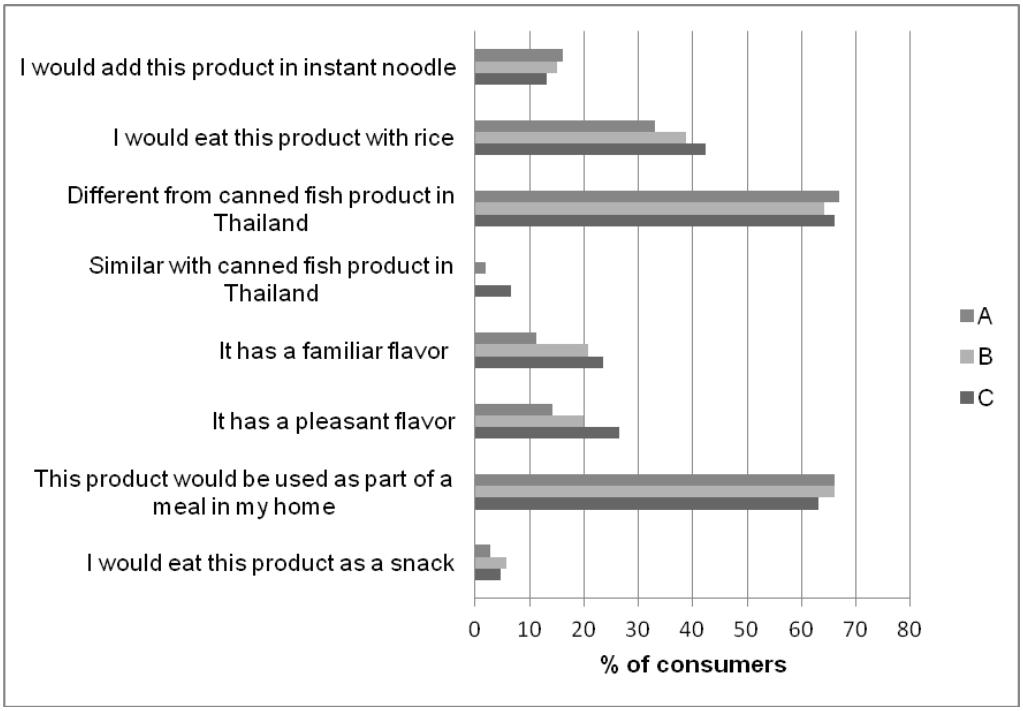

Table 1 Samples tested and their technological properties

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Sample	Curing time	Product appearance	Spiciness	Marination	Medium	Package	Shelf-life, months
A	10 days	Whole fish	Moderate (1.7g/100g fish)	No	Salt brine	Metal tin, 100g	3
B	2 days	Fish fillets	Mild (1.0g/100g fish)	No	Vacuum	Vacuum bag, 100g	1.5
C	5 days	Fish fillets	High (2.5g/100g fish)	Yes	Vegetable oil	Glass jar, 200g	3

Table 2 Attributes, definitions, and reference materials used in descriptive sensory analysis

Table 2 Attributes, definitions, and reference materials used in descriptive sensory analysis

Attribute	Definition	Reference material and intensity
Shininess	Degree of skin shine from dull to shiny	Picture *
Meat Color	Meat color intensity from light to dark	Picture *
Overall spiciness	Overall spiciness intensity	Seasoning mix = 12.0 **
Allspice	Allspice flavor intensity	Spice grains = 9.5
Nutmeg	Nutmeg flavor intensity	Ground nutmeg = 13.0
Nutmeg Flower	Nutmeg flower flavor intensity	Ground nutmeg flower = 10.0
Cinnamon	Cinnamon flavor intensity	Ground cinnamon = 13.0
Bay leaves	Bay leaves flavor intensity	Ground bay leaves = 12.0
Black Pepper	Black pepper flavor intensity	Ground black pepper= 12.0
Vanilla	Vanilla flavor intensity	Vanilla sugar = 7.0
Clove	Clove flavor intensity	Clove grains = 12.0
Ginger	Ginger flavor intensity	Ground ginger = 10.0
Coriander	Coriander flavor intensity	Ground coriander = 10.0
Cardamom	Cardamom flavor intensity	Ground cardamom = 13.0
Fish	Characteristic fish flavor intensity	Dried Alaska Pollack = 9.0
Hardness	Hardness of fish meat; strength that is needed to cut completely through fish meat with molars.	Sausage „Laste“= 7.0
Moistness	Moistness of fish meat; evaluated as pressing the meat against palate with tongue.	Canned meat „Turisti eine“ = 5.0
Greasiness	Greasiness of fish meat; evaluated from greasy mouthcoating that results from chewing.	Canned meat „Turisti Eine“ =10.0
Sweet	Basic taste, characterized by sucrose water solution.	0,3% sucrose = 3.0 0,5% sucrose =6.0 0,7% sucrose = 9.0 0,9% sucrose= 12.0
Sour	Basic taste, characterized by citric acid water solution.	0,025% citric acid = 2.5
Salty	Basic taste, characterized by sodium chloride water solution.	0,35% NaCl = 5.0 0,5% NaCl = 7.5 0,7% NaCl = 10.0 1,0% NaCl =12.0
Rancid	Off-flavor that results from fatty acid oxidation	5 min heated rapeseed oil = 8.0

*Picture references not shown

**Commercial spice mixture

All of the dried seasonings were measured in the amount of 5 ml individually into medium-sized sniffing glasses, covered with watch glasses

Table 3 Consumers and consumer clusters, demographic information (%)
Estonia – EST, clusters EST1, EST2; Thailand – THAI, clusters THAI1, THAI2

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Estonia – EST, clusters EST1, EST2; Thailand – THAI, clusters THAI1, THAI2.

	Country/clusters	EST	EST1	EST2	THAI	THAI1	THAI2
Gender	Male	37	37	36	36	33	50
	Female	63	63	64	64	67	50
Age	18-24	20	25	14	58	59	55
	25-35	45	45	42	27	29	18
	36-45	19	12	28	9	6	23
	46-55	8	7	8	5	5	5
	56-65	6	8	12	1	1	0
	66 or older	2	2	2	0	0	0
Education	High school or less/High school	13	70	7	27	27	30
	Some college/College degree	38	20	47	72	73	70
	Graduate degree/ Professional degree	49	10	46	1	0	0
Fish consumption frequency	Daily	2	3	0	7	6	9
	Several times/week/ At least once/week	73	69	76	67	64	68
	Several times/month	22	22	22	10	13	0
	Less often	4	3	2	16	13	23
Fish purchase	Supermarket	69	58	58	43	46	46
	Market	26	28	24	44	46	59
	Weekend market	6	7	6	18	23	13
	Other	12	18	10	9	6	0
Fish consumption	Fresh fish/fillet	50	45	53	33	35	14
	Frozen fish	38	42	34	15	16	23
	Salted/dried fish	22	20	24	24	26	27
	Canned fish	47	83	80	63	10	5
	Surimi products	56	63	48	20	21	23
	Fried fish/cakes	32	32	31	72	74	73
	Cured fish	82	83	80	8	10	5

* The percentage of consumers is calculated according to cluster size

Table 4 Water, lipid, protein and NaCl content of the samples ± stdev

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Sample	Water %	Lipid %	Protein %	NaCl %
A	61.2±0.10	12.6±0.88	13.5±0.13	8.6±0.03
B	63.8±1.14	12.2±2.32	11.8±0.35	6.0±0.39
C	57.2±0.97	16.3±0.13	12.1±0.70	5.6±0.15

Table 5 Flavor description of the spice-cured sprat products tested

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Attribute/Sample	A	B	C
Shininess	6.4 a	6.2 ab	4.6 b
Meat Color	7.3 a	4.6 c	5.7 b
Overall spiciness	5.7 a	3.3 b	5.5 a
Allspice	2.9 a	1.9 a	2.7 a
Nutmeg	0.7 a	0.2 a	0.7 a
Nutmeg Flower	0.3 a	0.0 b	0.1 ab
Cinnamon	0.3 a	0.0 a	0.3 a
Bay leaves	1.8 a	1.0 a	1.3 a
Black Pepper	1.7 a	1.4 a	2.0 b
Vanilla	0.1 a	0.1 a	0.1 a
Clove	0.7 a	0.3 a	0.8 a
Ginger	ND	ND	ND
Coriander	0.4 a	0.03 a	0.4 a
Cardamom	0.03 a	0.0 a	0.03 a
Fish	10.2 a	10.6 a	8.7 b
Hardness	6.3 a	5.0 b	5.6 ab
Moistness	5.6 a	6.6 a	6.5 a
Greasiness	6.1 a	6.5 a	6.9 a
Sweet	3.6 a	3.5 a	3.6 a
Sour	0.6 b	0.5 b	2.0 a
Salty	11.4 a	9.5 b	9.4 b
Rancid	0.8 a	1.2 a	0.7 a

ND – not detected; cells with different letters in a row are significantly different ($p<0.05$)

Table 6 Mean values, ANOVA and JAR results for consumer acceptance in Estonia and in Thailand. EST-Estonia; THAI-Thailand

Table 6 Mean values, ANOVA and JAR results for consumer acceptance in Estonia and in Thailand. EST-Estonia; THAI-Thailand

	Mean score EST	Groups EST	JAR levels (collapsed) %consumers EST			Mean score THAI	Groups THAI	JAR levels (collapsed) %consumers THAI		
			Too little	JAR	Too much			Too little	JAR	Too much
APPEARANCE										
A	6.7	a	NA	NA	NA	5.2	a	NA	NA	NA
B	6.1	b	NA	NA	NA	5.3	a	NA	NA	NA
C	5.5	c	NA	NA	NA	5.3	a	NA	NA	NA
AROMA										
A	6.5	a	NA	NA	NA	4.5	b	NA	NA	NA
B	6.0	b	NA	NA	NA	4.8	b	NA	NA	NA
C	5.6	c	NA	NA	NA	5.7	a	NA	NA	NA
FLAVOR										
A	6.5	a	14.5	41.8	44.5	4.4	b	6.6	16.0	77.4
B	6.1	a	14.5	40.0	46.4	4.8	b	8.5	33.0	58.5
C	5.4	b	15.5	22.7	62.7	5.4	a	4.7	33.0	62.3
FISH FLAVOR										
A	6.4	a	23.6	54.5	22.7	4.4	b	5.7	24.5	69.8
B	6.0	b	22.7	48.2	30.0	4.9	b	10.4	26.4	63.2
C	5.4	c	46.4	25.5	29.1	5.4	a	7.5	40.6	51.9
SALT										
A	5.8	b	7.3	33.6	60.0	3.7	b	4.7	10.4	84.9
B	5.8	a,b	5.5	46.4	49.1	4.4	a	13.2	22.6	64.2
C	5.4	a	9.1	29.1	62.7	4.6	a	11.3	22.6	66.0
AFTERTASTE										
A	6.4	a	10.9	56.4	32.7	4.6	b	5.7	32.1	61.3
B	6.0	a	10.0	59.1	30.9	4.8	b	6.6	35.8	57.5
C	5.2	b	8.2	41.8	50.0	5.3	a	5.7	39.6	54.7
TEXTURE										
A	6.6	a	10.9	65.5	24.5	4.6	a	61.3	32.1	6.6
B	6.0	b	63.6	30.0	7.3	4.7	a	73.6	24.5	1.9
C	5.4	c	60.9	30.9	8.2	5.1	a	77.4	18.9	3.8
OVERALL LIKING										
A	6.6	a	NA	NA	NA	4.6	b	NA	NA	NA
B	6.1	b	NA	NA	NA	4.8	b	NA	NA	NA
C	5.5	c	NA	NA	NA	5.4	a	NA	NA	NA
WOULD BUY*										
A	3.2	a	NA	NA	NA	2.5	b	NA	NA	NA
B	2.5	b	NA	NA	NA	2.8	a,b	NA	NA	NA
C	2.3	b	NA	NA	NA	3.0	a	NA	NA	NA

* 5-point scale; NA-not assessed; cells with different letters for an attribute in a column are significantly different ($p<0.05$).

Table 7 Number of consumers, most frequently liked/disliked products (A, B, C), mean values, ANOVA, number and percentage of consumers who were also grouped in the strict, strict liking only, loose or loose liking only clusters for AHC clusters of Estonian (EST1, EST2) and Thai (THAI1, THAI2) spice-cured sprat products study

Table 7 Number of consumers, most frequently liked/disliked products (A, B, C), mean values, ANOVA, number and percentage of consumers who were also grouped in the strict, strict liking only, loose or loose liking only clusters for AHC clusters of Estonian (EST1, EST2) and Thai (THAI1, THAI2) spice-cured sprat products study

Cluster	Number of members	Liked products, mean	Neither liked or disliked products, mean	Disliked products, mean	Strict		Strict liking only		Loose		Loose liking only	
					Nr.	%	Nr.	%	Nr.	%	Nr.	%
EST1	60	A 6.5a	C 6.4a	B 5.3b	25	42	31	52	36	60	36	60
EST2	50	B 7.0a	A 6.8a	C 4.3b	34	68	34	68	46	92	46	92
THAI1	82	C 5.6a	B 5.4a	A 5.3a	24	29	38	46	50	61	50	61
THAI2	22	C 4.5a	B 2.6b	A 2.0b	13	59	17	77	17	77	17	77

Liked products = the most frequently high rated by consumers for the best liked products.

Neither liked nor disliked products = products rated nor highest or lowest by consumers.

Disliked products = the most frequently low rated by consumers for least liked products.

If letters are different within country for a sample (a, b), the differences are statistically significant ($p<0.05$).

PUBLICATION IV

Timberg L, Jakimtšuk A, Viiard E, Koppel K, Kuldjärv R, Sarand I, Paalme T

The effect of freezing-thawing and packaging material on the microbial dynamics of spice-cured sprats

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The effect of freezing-thawing and packaging material on the microbial dynamics of spice-cured sprats

Journal of Applied Microbiology

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Running headline: Microbial dynamics of cured sprats

Abstract

Aims: To evaluate the effect of microbiota on sensory and quality properties of spice-cured sprats, and to determine freezing-thawing and oxygen availability impact on a product shelf-life.

Methods and Results: The microbial composition and sensory properties of spice-cured sprats were analyzed over 12 weeks of curing using Rep-PCR fingerprinting, 16S rRNA gene sequencing, and descriptive sensory analysis. Preparations of fresh and frozen-thawed fish both packed into glass jars and plastic containers were analyzed. Spice-cured sprats were dominated by salt tolerant species *Brochothrix thermosphacata* and *Lactobacillus sakei.* When fresh fish was used as a raw material *Aerococcus viridans* also dominated. Plastic containers promoted the growth of *Aerococcus viridans* and *Lactobacillus sakei* relative to glass jars and increased the rate of spoilage. We explain this by the additional oxygen that permeates through plastic.

Conclusion: Freezing-thawing of fish was found to have a negative effect on the plate count and delayed the microbial growth during curing. The role of microorganisms in the ripening of spice-cured sprats is small but can be significant in spoilage.

Significance and Impact of the Study: To obtain a high and stable quality spice-cured sprat product a fresh fish and air-tight package materials would be recommended.

Keywords: baltic sprat, fish, lactic acid bacteria, ripening, sensory

Introduction

Spice-cured sprat is a salted and spiced ready-to-eat fish product, which is a traditional product in Eastern Europe. Spice-cured sprats are produced mostly in the Baltic countries and the annual volume is about 35 thousand tonnes, which is 40% of the whole Baltic countries sprat catch. Spice-cured sprats became popular already in the 1930s and were nominated as the best fish product at the Brussels Seafood competition. Today they are listed in the book "1001 Foods You Must Taste Before You Die" (Case, 2008). The core production scheme and spice mixture of spice-cured sprats has not changed, however modern pretreatment and packaging options are now used. Spice-cured sprats are made from whole fish, which have been layered into jars with a mixture of spices and covered with salt brine. They develop their characteristic properties during ripening. Originally spice-cured sprats were mainly prepared from fresh sprats caught during autumn and winter, because of the best sensory properties and highest fat content 15-23% (Krosing and Veldre, 1973). Currently, spice-cured sprats are produced all year round from both fresh and frozen-thawed sprats using even spring catches with low fat content (10%) (Timberg *et al*., 2011). The sensory properties and shelf-life of spice-cured sprats could also differ from traditional recipes due to the use of permable to oxygen plastic package materials.

Lightly processed and refrigirated fish products contain NaCl, which inhibits the growth of Gram-negative, pH-sensitive psychrotrophic bacteria naturally present in fish (e.g. *Pseudomonas* spp.), and allows the growth of other organisms more resistant (lactic acid bacteria, *Micrococecae, Enterobacteriaceae, Brochothrix thermosphacata)* (Truelstrup Hansen, 1995; Lyhs *et al*. 2007; Leroi, 2010). Lactic acid bacteria (LAB) have been identified as dominant microorganisms in many lightly processed and refrigirated fish products (e.g. salted herring and spice-cured sprats) (Stenström, 1985; Hong *et al*., 1996; Emborg *et al*., 2002; Dabrowski *et al*., 2001; Dalgaard *et al*., 2003; Lyhs and Björkroth, 2008; Leroi, 2010). Lactobacilli species have also been isolated from the intestinal tract of fish (Kraus 1961). LAB communities within fish products affect their shelf life and the growth of LAB may vary depending on the pretreatment of the fish, the processing

technology used, and the packaging. Lyhs *et al*. (2007) detected 10^2-10^4 and 10^6 CFU g-1 of LAB in sensorial spoiled maatjes herring stored at 4ºC and 10°C, respectively. *Lactobacillus sakei* and some other *Lactobacillus* species produce characteristic off-odours associated with spoiled vacuum-packed cold-smoked salmon (Truelstrup Hansen, 1995; Joffraud *et al*., 2001; Leroi *et al*., 1996, 1998; Paludan-Müller *et al*., 1998).

The genus *Carnobacterium* belongs to the natural microbiota of both fresh and frozen-thawed fish, and can affect the sensory properties (Ringo *et al*., 2010; Michel *et al*., 2007; Pons-Sánchez-Cascado *et al*., 2005). The prominent representatives of *Carnobacterium* species, particulary in frozen and thawed seafood products, are *Carnobacterium divergens* and/or *Carnobacterium maltaromaticum* (Leisner *et al*., 2007). *Carnobacterium* species can produce antimicrobial peptides, bacteriocins (e.g. Lewus *et al*., 1991; Coventry *et al*., 1997), and cause glucose depletion (Leroi *et al*., 1996; Buchannan and Bagi, 1997; Nilsson, *et al*., 1999, 2005). These observations suggest that the presence of some *Carnobacterium* species in foods can have a positive effect on the shelf-life of lightly preserved seafood (Einarsson and Lauzon, 1995; Roller *et al*., 2002; Lauresen *et al*., 2006). Previous studies have shown that *C. maltaromaticum* and *C. divergens* may produce metabolites, such as tyramine, ammonia, ornithine, acetate, diacetyl/acetoin, and methylbutanol, which can cause off-odors (Lauresen *et al*., 2006), but have also been observed to have no effect on off-odours/flavours (Joffraud *et al*., 2001). Leisner and colleagues (2007) found that although carbohydrate catabolism by *Carnobacterium* species appears to result in a diverse number of metabolites, these generally have a limited effect on the sensory attributes of foods.

The aims of this study are to 1) identify the dominating bacteria in sprats and spice-cured sprats made from fresh and frozen-thawed fish and packed into glass or plastic jars, and 2) to identify the relationships between dominating species, sensory characteristics, and product spoilage. To the authors' knowledge this is the first study to identify and quantify bacterial strains isolated from spice-cured sprats and evaluate the effect of microbial growth on sensory properties. This study builds upon work to develop a sensory lexicon of spice-cured sprat products with an evaluation of

their market potential Timberg *et al*., 2012 (in print), and a review of how the chemical, physical, and sensory properties develop in spice-cured sprat Timberg *et al*., 2012 (in print).

Materials and Methods

Spice-Cured Sprats

Fifty kilograms of Baltic sprat was obtained from a local fisherman in November 2010. Half of the fish were spice cured the day after catching, and half of the fish were frozen. Prior to freezing the fish were packed into zip-lock plastic bags (final weight 3.5 kg, dimensions 45 × 35 × 3 cm). The bags were frozen at -40°C for 24 hours and then transferred into a freezer at -18°C. Spice-curing was carried out with a traditional spice mixture (1.7 g / 100g of fish), containing several different spices including vanilla, cinnamon, cardamom, coriander, ginger, nutmeg, nutmeg flower, allspice, clove, black pepper, and bay leaves (produced by Paulig Estonia). Whole sprats (including heads and guts) were layered into glass and plastic jars (polypropylene, O_2 barrier 1550 ml/m^2/24h, CO_2 barrier 8800 ml/m^2/24h, N_2 barrier 640 ml/m^2/24h, volume 185 ml) together with the spice-mix, and covered with brine (23% NaCl solution), and sealed with a metal lid (glass jars) or a plastic lid (plastic jars). The ratio of fish to brine in all preparations was 70:30. Preserved sprats were cured at 4°C and analysed at different times between one to twelve weeks. Frozen sprats were kept at -18°C for three months, after which they were thawed at 4°C during 24 hours, and cured in glass and plastic jars using the same method as the fresh fish. These procedures correspond to those applied in the industrial production of spice-cured sprats.

Microbial Analysis

A volume of 70 ml of 0.85% NaCl solution was added into a Stomacher bag after adding the equivalent of one tin of whole fish (175g fresh or thawed). The mixture was homogenized for 5 minutes at 230 rpm in a Stomacher 400 circulator (Seward Ltd., UK). Decimal dilutions were made from the fish mixture and plated on MRS (de Man *et al*., 1960), MRS + 7% NaCl, and PCA (plate count agar) media (media obtained from LabM Ltd., UK). The plates were incubated under

anaerobic conditions at 22°C for 3 days. In order to count and isolate species characteristics, plating was done with MRS, PCA, and MRS+7% NaCl agars for fresh fish, frozen-thawed fish, and sprats cured both in glass and plastic jars.

DNA Extraction

Selected colonies (10-20 from each sample point) were picked from three plates and grown in liquid media. FTA MiniCards (GE Healthcare Ltd., UK) were used for DNA extraction of the isolates according to the manufacturer's instructions. 30 µl of liquid culture was pipetted onto the FTA card and allowed to dry for one hour. A 1.2 mm^2 piece was cut from the card and placed into a 250 µl sterile tube. It was washed twice with the FTA washing solution (150 µl) and twice with TE-buffer (also 150 µl). The washed FTA paper was dried at 50 °C for 15 minutes and then used for repetitive element palindromic polymerase chain reaction (Rep-PCR) analysis.

Repetitive Element Palindromic-PCR

Rep-PCR with primer $(GTG)_5$ was performed essentially as described by De Vuyst *et al*., 2002. The PCR reaction was carried out in 25 µl using the following cycle: preliminary denaturation for 6 minutes at 95 °C; amplification in 30 cycles: denaturation at 94 °C for 1 minute, annealing for 1 minute at 40 °C, extension for 8 minutes at 65 °C and final extension at 65 °C for 16 minutes. The fragments were visualized in a 1% agarose gel using a Bio-Rad gel electrophoresis system (Bio-Rad Laboratories, Inc., USA). A Bionumerics 6.0 platform (Applied Maths NV, Belgium) was used for fingerprint analysis.

16S rRNA Gene Sequencing

The main bacterial species in sprat samples were identified using Rep-PCR fingerprinting and 16S rRNA gene sequencing. 16S rRNA gene fragments were amplified using universal primers 27f-YM (Frank *et al*., 2008) and 16R1522 (Weisburg *et al*., 1991). The fragments were purified with GeneJET PCR Purification Kit (Fermentas, Lithuania) and prepared for a PCR sequencing reaction using BigDye Terminator v3.1 Cycle Sequencing Kit as described by manufacturer (Applied

Biosystems, USA). The partial 16S rRNA sequences were compared using the BLAST algorithm together with the GenBank database (National Center for Biotechnology Information, USA) to find the close matches.

Descriptive Sensory Analysis

A trained panel of six panellists was used for descriptive sensory analysis to describe the spice-cured sprat products. All of the panellists had previous experience in descriptive sensory analysis with various food products including at least 50 h evaluation of seasoned sprats. All were employees of the Competence Centre of Food and Fermentation Technologies in Tallinn, Estonia. The panellists were further trained for this test during five sessions lasting 1.5 hours each, where the attributes, definitions and reference materials were agreed upon. During these sessions the panellists had access to commercial samples with similar attributes to the test samples. Samples were described by their appearance, flavour and texture attributes using the method presented by Timberg *et al*. (2012, in print). Shininess and meat colour were two attributes used for appearance evaluation. Seventeen attributes were used for flavour and taste evaluation: overall spiciness, allspice, nutmeg, nutmeg flower, cinnamon, bay leaves, black pepper, vanilla, clove, ginger, coriander, cardamom, fish, sweet, sour, salty, and rancid. Three attributes were used for texture evaluation: hardness, moistness, and greasiness.

All samples were evaluated in triplicate. The sensory laboratory was equipped with individual booths and computers according to ISO 8589-2007. The panellists used a data collection program, written internally, to enter scores. A scale with 0.5 point increments, where 0 = none and 15 = very strong, was used. Unsalted crackers and purified filtered water was available at all times, along with reference materials and definition sheets. The panellists were reminded to clean their palates in between the samples. The samples were served on white ceramic plates, coded in three-digit numbers. Each panellist was served two whole fish for evaluation. The serving of the samples was randomized. The samples were prepared 30 minutes prior to evaluation, while references were

prepared 30 minutes to two hours in advance. The samples were stored at 2-6 °C prior to evaluation.

pH Analysis

pH content of the samples were measured in triplicate and the results were averaged. All samples (ca 300 g of fish) were filleted, and drained off excess fluids and minced. For pH measurements the minced fish was diluted with distilled water (1:10) and homogenized, and measured with 744 pH Meter, Metrohm, Switzerland.

Data Analysis

A 2^2 factorial design was applied in the present study. The two variables, or factors, studied were freezing-thawing and package material. XL Stat version 2011.1.04 (XL Stat, New York, NY, USA) was used for data analysis. Analysis of Variance (ANOVA) was performed for plate counts and sensory analysis results and significant differences (p=0.05) among samples were found using Fisher's protected least significant difference (LSD).

Results

Fresh versus Frozen-Thawed Sprats

Fresh sprats were found to have plate counts of 10^3 CFU g-1, while frozen-thawed sprats had plate counts of 10^2 CFU g-1. One magnitude lower plate counts for frozen-thawed sprats were typical for different batches over a number of years (data not shown). Figure 1 shows that the fingerprint types for bacteria isolated from sprats, frozen-thawed and spice-cured sprat products display a high variability. MRS isolates from fresh sprats displayed 8 different fingerprint types corresponding according to 16S rRNA sequencing to species of *Carnobacterium divergens, Carnobacterium inhibens, Carnobacterium maltaromaticum, Carnobacterium* sp., *Brochothrix thermosphacata*, *Enterococcus* sp., *Hafnia alvei* and *Vagococcus salmonarium*. Fewer species and genera were

detected in frozen-thawed sprats - one fingerprint type of *Carnobacterium* sp., *Enterobacter clocae*, *Lactobacillus sakei* and *Vagococcus salmoninarum*. Interestingly, the fingerprint types found in fresh and frozen-thawed fish did mostly not correspond to those of cured sprats prepared from same material.

Spice-Cured Sprats in Glass Package

Although present in low proportion in fish *Brochothrix thermosphacta* was dominant at week 4 of sprat curing, both in samples made from fresh (Table 1) and frozen-thawed fish (Table 2). After further curing its proportion decreased. *Lactobacillus sakei* was the second species found in high numbers in cured sprats. Spice-cured sprats made from fresh fish and packed into glass jars at week 8 were dominated by *Brochothrix thermosphacata* and *Lactobacillus sakei*, and both had plate counts higher than 10^6 CFU g-1 (Table 1). Surprisingly, *Lactobacillus sakei* was not detected in samples made from fresh fish at week 12. Instead, *Staphylococcus euquorum* was found in concentrations of 10^5 CFU g-1. The effect can be explained by very low numbers of both species in fresh fish and heterogeneity of material which can cause variation between batches. In spice-cured sprats made from fresh fish we found also *Aerococcus viridans* and *Wiesella hellenica* and in samples made from frozen-thawed fish *Carnobacterium* spp. and *Lactobacillus curvatus* on the MRS plates but not on MRS + 7% NaCl plates. The results show also that the growth intensity of microorganisms was delayed in sprats made from frozen-thawed fish, probably due to lower initial plate counts.

Spice-cured Sprats in Plastic Package

During week 4 it was observed that products cured in plastic jars had higher numbers of colony forming bacteria, regardless of whether fresh or frozen-thawed fish were used for curing (Table 1, Table 2). The salt tolerant *Lactobacillus sakei* was detected in samples made from fresh fish together with *Aerococcus viridans* and *Carnobacterium* spp., and in samples made from frozen-thawed fish these species were found together with *Brohotrix thermosphacta* and *Staphylococcus*

euquorum. During four weeks in case of fresh fish and eight weeks in case of frozen-thawed fish plate counts of spice-cured sprats in plastic containers reached 10^6 CFU g-1. This is a typical threshold for the number of bacteria required to influence the sensory properties of food (Leisner *et al*., 2007; Laursen *et al*., 2006; Chenoll *et al*., 2007; Lyhs and Björkroth, 2008). In spice-cured sprats made from fresh fish *Lactobacillus sakei* and *Aerococcus viridans* were dominant, but also *Carnobacterium* spp. and *Brohotrix thermosphacta* were detected at week 8-12. In spice-cured sprats made from frozen-thawed fish, *Lactobacillus sakei* and *Brohotrix thermosphacta* were dominant, but also *Bacillus subtilis* was detected.

Sensory Properties Development of Spice-Cured Sprats

The salt content of the sprats and brine equilibrated within the first four weeks in both fresh and frozen-thawed fish samples. Thereafter, the salt concentration (6% in fish) remained constant for the remainder of the ripening period. There was a tendency that the pH of spice-cured sprats was lower in glass jars (from 6.63±0.02 to 6.59±0.01 at weeks 2 and 12, respectively) compared to plastic containers (from 6.73±0.01 to 6.62±0.03 at weeks 2 and 12, respectively) (p=0.05).
The most important sensory attributes of spice-cured sprats describe the changes that occur during ripening were sour flavour, rancid flavour, and hardness. Sour and rancid flavour scores were interpreted as follows: 0-0.50 weak sour/rancid; 0.51-1.00 sour/rancid; 1.01< strong sour/rancid (Table 1, Table 2). Spice-cured sprat samples packed into plastic containers had a tendency to be more sour and a rancid flavour developed more rapidly than in samples packed into glass jars.

Discussion

The increasing sour and rancid flavour scores with increasing curing time is likely a result of enzymatic activity. Sour and rancid taste increased also with number of microorganisms. However, no statistically significant correlation between sour flavour and total viable cell count

was found, probably because of the variability of the raw material (heterogeneity of fish schools microflora) and autolysis of bacteria.

The numbers and patterns of species found using MRS and PCA agar plates of fresh and frozen-thawed sprats were similar and therefore in curing studies MRS and MRS+7%NaCl were used. The observed patterns of species show that *Brochothrix thermospacta*, which is detected in fresh sprats, is also present in all spice-cured sprat samples made from fresh and frozen-thawed fish when packed into both glass jars and plastic containers. The other species that reached plate counts over 10^6 CFU g-1 in spice-cured sprats (*Aerococcus viridans, Staphylococcus equorum* and *Lactobacillus sakei*) were not detected in fresh or frozen-thawed sprats, probably due their low percentage and low plate counts ($<10^3$ CFU g-1) .

One of these, *Aerococcus viridians*, was present in sufficient numbers to cause spoilage only in spice-cured sprats made from fresh fish and packed into plastic containers. *Aerococcus* spp. is known to be aerophilic (Williams *et al*., 1953) and the high plate counts indicate that these species might be the main cause of premature spoilage of spice-cured sprats preserved in plastic containers, which have a higher oxygen permeability. *Aerococcus viridians* were not detected in spice-cured sprats made from frozen-thawed fish, which suggests that freezing has a negative effect on the culturability and viability of these bacteria.

Lactobacillus sakei was the second species that reached high colony forming numbers ($>10^6$ CFU/g), commonly at week 8. *Lactobacillus sakei/curvatus* have been reported to be major spoilage bacteria in chilled seafood (Lyhs and Björkroth, 2008) and meat products (Björkroth and Korkela, 1997). In plastic containers, high numbers of *Lactobacillus sakei* were observed earlier (week 4). It is possible that the combined presence of species belonging to the genus *Aerococcus* and oxygen, promote the growth of *Lactobacillus sakei* in sprats spice-cured in plastic containers.

In spice-cured sprats produced using frozen-thawed fish, the *Lactobacillus sakei* count remained lower than in samples produced using fresh fish. It has been suggested that during the ripening of fish products made without removing intestines, LAB migrate from the intestines to the muscle tissue and become dominant later due to packaging and storage conditions (Lyhs and Björkroth, 2008). *Lactobacillus sakei* is known to proliferate at low water activities and tends to develop sour and sulphuric odors and flavours in food products because of its ability to produce H_2S and lactic acid (Truelstrup Hansen, 1995; Korkela and Björkroth, 1997; Samelis *et al*., 2000; Stohr *et al*., 2001; Samelis 2006). The acid note in spice-cured sprats correlates well with the plate count of *Lactobacillus sakei*, however, sulfuric flavour notes in spice-cured sprats were not detected. The growth of *Lactobacillus sakei* on MRS+7% NaCl agar was dominant because this species is known to have a high salt tolerance (Samelis *et al*., 2000; Samelis, 2006; Lorenzo *et al*., 2010).

The third prevailing organism in cured sprats was *Brochotrix thermosphacata* which is known to be a moderate spoilage organism. Oxygen availability increases both the spoilage potential and the number of metabolites produced by *Brochotrix thermosphacata* (Laursen *et al*., 2006). *Brochothrix thermosphacata* is also known to produce acetoin, diacetyl, 2,3-butandiol, acetic acid, isobutyric acid, isovaleric acids, 2-heptanone, and 2-hexanone, which result in an unpleasant sour, butter, cheesy, blue-cheese, pungent, and sweaty feet off-odours (Joffraud *et al*., 2001; Mejlholm *et al*., 2005). Off-odours are particularly offensive when oxygen is present (Leroi *et al*., 1998; Joffraud *et al*., 2001; Pin *et al*., 2002). In sprats cured in plastic containers, more oxygen was available, however, under those conditions, other species (*Lactobacillus sakei* and *Aerococcus viridans)* outgrew *Brochotrix thermosphacata*. That, together with a relatively low count and effect of species, might explain why the off-odors characteristics to *Brochotrix thermosphacata* were not detected in our sensory analysis of over-ripened sprats. Another possible explanation is that some of these off-odours, in combination with the spice mixture, were interpreted as characteristic odours of spice-cured sprats.

The growth of *Carnobacterium* spp. during curing of sprats was low compared to other species (e.g. *Aerococcus viridans, Lactobacillus sakei* and *Staphylococcus equorum*) and did not produce detectable off-odors. Also, Leisner *et al* (2007) found that in naturally contaminated products with *Carnobacterium* spp. other members of the microbial community are typically more important with regard to sensory effects, including spoilage.

The spice-cured sprats obtained optimal sensory properties after 4 weeks of storage, during which time the microbial concentrations tended to remain low enough to avoid influencing the sensory properties. Thus, we can conclude that the role of microorganisms in the ripening of spice-cured sprats is small. After 8 weeks, however, the microbial concentration of *Lactobacillus sakei, Aerococcus viridans* and/or *Staphylococcus equorum* spp. reached a level that may negatively affect the sensory properties by causing both a strong sour taste and a rancid flavour. Alternative preservation strategies could be adopted to prolong sensory shelf-life. Storage at a sub-zero temperature (-2 ºC), might prevent the growth of microorganisms and prolong the storage time, or alternatively biopreservation could be applied using microorganisms who are antagonistic for the spoilage bacteria (Jameson, 1962; Ross and McMeekin, 1991; Gimenez and Dalgaard, 2004).

We found that the dominating species in fresh Baltic sprats *Carnobacterium* spp., *Vagococcus salmoniarum* sp., *Enterococcus* spp., *and Halfia alvei* sp. are detected independent of the growth media used (MRS and PCA) and they are replaced during the curing of sprats by *Lactobacillus sakei*/*curvatus, Aerococcus viridans*, and *Brochothrix thermosphacata, which* reached levels (>10^6 CFU g-1) that could change the sensory properties and accelerate spoilage. Freezing-thawing of fish will delay the growth intensity of microbiota and change the patterns of species observed in spice-cured sprats. When cured in plastic containers (not hermetic), aerophilic organisms such as *Aerococcus viridans* may contribute to the spoilage of spice-cured sprats. Overall, the role of microorganisms in the ripening of spice-cured sprats is small while in spoiling it can be significant.

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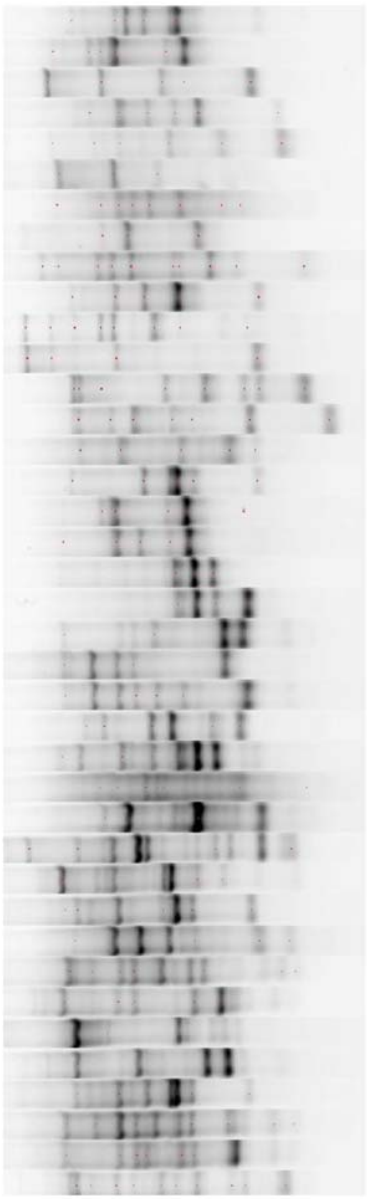

Group	Genus	Species	Origin
C5	Brochothrix	thermosphacta	F
F1	Carnobacterium	divergens	F
F2	Carnobacterium	inhibens	F
A4	Carnobacterium	maltaromaticum	F
A8	Carnobacterium	sp.	F
G2	Enterococcus	sp.	F
K1	Hafnia	alvei	F
E1	Vagococcus	salmoninarum	F
N1	Aerococcus	viridans	FG
C2	Brochothrix	thermosphacata	FG
A3	Carnobacterium	funditum	FG
A9	Carnobacterium	sp.	FG
D1	Staphylococcus	equorum	FG
D2	Staphylococcus	equorum	FG
M1	Weisella	hellenica	FG
C1	Brochothrix	thermosphacata	FP
A1	Carnobacterium	divergens	FP
A2	Carnobacterium	divergens	FP
A5	Carnobacterium	sp.	FP
A6	Carnobacterium	sp.	FP
B2	Lactobacillus	sakei	FP
B3	Lactobacillus	sakei	FP
B7	Lactobacillus	sakei	FP
J1	Micrococcus	sp.	FP
A7	Carnobacterium	sp.	Fr
H1	Enterobacter	cloacae	Fr
E2	Vagococcus	salmoninarum	Fr
L1	Bacillus	licheniformis	FrG
I1	Bacillus	subtilis	FrG
C3	Brochothrix	thermosphacata	FrG
G1	Enterococcus	hermannensis	FrG
B1	Lactobacillus	curvatus	FrG
B6	Lactobacillus	sakei	FrG
D4	Staphylococcus	pasteuri	FrG
D5	Staphylococcus	pasteuri	FrG
C4	Brochothrix	thermosphacata	FrP
B4	Lactobacillus	sakei	FrP
B5	Lactobacillus	sakei	FrP
D3	Staphylococcus	equorum	FrP

Fig. 1. The closest relative for sequences obtained from 16S rRNA gene of spice-cured sprat samples made from fresh and frozen fish and packed into glass and plastic package in BLAST GenBank. Fresh fish (F), Frozen-thawed fish (Fr), spice-cured sprats from Fresh fish in Glass jars (FG), spice-cured sprats from Fresh fish in Plastic jars (FP), spice-cured sprats from Frozen-thawed fish in Glass jars (FrG), spice-cured sprats from Frozen-thawed fish in Plastic jars (FrP).

Table 1. Bacterial counts (log CFU g^{-1}), the different obtained taxa, sensory characteristics and pH of spice-cured sprat samples made from fresh fish and packed into glass and plastic package.

	Fresh	Fresh glass			Fresh plastic			
		4 weeks	8 weeks	12 weeks	1 week	4 weeks	8 weeks	12 weeks
MRS	3.5±0.2	3.5±0.1	6.2±1.0	5.4±0.5	3.5±1.0	6.3±0.7	4.4±0.2	6.6±0.9
PCA	3.9±0.3	NT	NT	NT	3.3±0.9	NT	NT	NT
MRS+7%NaCl	NT	NT	6.3±0.2	5.1±0.3	3.0±0.4	6.0±1.2	7.5±0.0	6.3±0.8
Aerococcus viridans sp.		9%	18%			12%/12%**	39%/50%**	31%/33%**
Brochothrix thermosphacta sp.	7%	91%	59%	25%	55%	6%	8%	8%
Carnobacterium divergens sp.	7%					12%	15%	15%
Carnobacterium funditum sp.				9%				
Carnobacterium inhibens sp.	7%				9%			
Carnobacterium maltaromaticum	7%							
Carnobacterium spp.	7%/100%*			16%	9%	6%	8%	
Enterococcus sp.	20%							
Hafnia alvei sp.	13%							
Lactobacillus sakei sp.			100%**		9%/100%**	64%/88%**	30%/50%**	46%/67%**
Micrococcus sp.					18%			
Staphylococcus equorum sp.				32%/100%**				
Vagococcus salmoninarum sp.	32%							
Weissella hellenica sp.			23%	18%				
pH	7.02±0.02	6.70±0.03	6.63±0.01	6.59±0.01	NT	6.75±0.01	6.63±0.02	6.62±0.02
Sensory characteristics	NT	Weak sour Weak rancid	Sour Rancid	Strong sour Strong rancid	NT	Weak sour Weak rancid	Strong sour Strong rancid	Strong sour Strong rancid

MRS- de Man, Rogosa and Sharpe, *PCA-Plate Count Agar, **MRS+7%NaCl, 3 parallel jars were analyzed at each sampling point, NT-not tested

Table 2. Bacterial counts (log CFU g^{-1}), the different obtained taxa, sensory characteristics and pH of spice-cured sprat samples made from frozen-thawed fish and packed into glass and plastic package.

	Frozen-thawed	Fozen-thawed glass				Frozen-thawed plastic			
		1 week	4 weeks	8 weeks	12 weeks	1 week	4 weeks	8 weeks	12 weeks
MRS	2.0±0.2	3.3±0.2	3.9±0.1	3.3±0.4	5.1±1.6	4.2±1.0	4.5±2.0	4.7±1.6	6.0±1.6
PCA	1.7±0.3	3.0±0.3	NT	NT	NT	4.1±1.1	NT	NT	NT
MRS+7%NaCl	NT	NT	2.0±0.0	NT	6.3±1.8	NT	2.4±0.9	5.7±0.2	5.7±1.6
Bacillus licheniformis sp.								12%	
Bacillus subtilis sp.								6%	
Brochothrix thermosphacta sp.		54%/100%*	64%	72%	19%	54%/100%*	82%	58%/50%**	18%
Carnobacterium divergens sp.	44%/82%*								
Carnobacterium inhibens sp.			12%	14%					
Carnobacterium spp.	17%	8%	18%	14%		15%			
Enterobacter cloacae sp.	21%/9%*								
Enterococcus hermanniensis		8%	6%						
Lactobacillus curvatus sp.					33%				
Lactobacillus sakei sp.	9%/9%*		100%**		48%/100%**		73%**	24%/50%**	82%/100%**
Staphylococcus equorum sp.							18%/27%**		
Staphylococcus pasteuri sp.						8%			
Vagococcus salmoninarum sp.	9%	30%				23%			
pH	6.86±0.01	NT	6.62±0.02	6.52±0.02	6.60±0.03	NT	6.65±0.02	6.62±0.05	6.60±0.03
Sensory characteristics	NT	NT	Weak sour Rancid	Sour Rancid	Strong sour Strong rancid	NT	Weak sour Rancid	Sour Strong rancid	Strong sour Strong rancid

MRS- de Man, Rogosa and Sharpe, *PCA-Plate Count Agar, **MRS+7%NaCl, 3 parallel jars were analyzed at each sampling point, NT-not tested

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Rainbow trout composition and fatty acid composition in Estonia

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Rainbow Trout Composition and Fatty Acid Content in Estonia

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Abstract. Rainbow trout (*Oncorhynchus mykiss*) is the most popular aquaculture species in Estonia. The aim of the present study was to examine and compare moisture, protein, lipid and fatty acid (FA) compositions in Rainbow trout from different fish farms in Estonia and that farmed in Finland and Norway. The total lipid content in different Rainbow trout varied more than 5.5 fold, but FA proportions were very similar in all Rainbowtrout. However, it is important to note that Estonian farmed Rainbow trout had generally lower lipid content and therefore also a lower amount of essential FAs.

Key words: Fatty acid, lipid, Rainbow trout (*Oncorhynchus mykiss)*

INTRODUCTION

Natural resources of fish can no longer fulfil demands of fish consumers; the shortage is forcing the aquaculture sector to expand. There are about 15 fish farms in Estonia where Rainbow trout is cultured. The annual volume of Estonian farmed Rainbow trout is about 700 tons, but many fish farms are expanding; production is expected to double in the next few years. Therefore, the Estonian aquaculture sector is interested in producing Rainbow trout which has high nutritional value, stabile quality and is also compatible with Rainbow trout farmed in other countries.

Fat is one of the most important components of fish meat. It attracts consumers' attraction due to the fatty acid (FA) profile, especially n–3 and n–6 FAs (Ruxton et al., 2004; Breslow, 2006). Therefore, the main aim of the study was to characterize and compare the moisture, protein, lipid and FA profiles of Rainbow trout from different fish farms in Estonia and imported Rainbow trout available in Estonian supermarkets.

Experiment design

Rainbow trout samples from ten different aquaculture facilities in Estonia were acquired (samples E1–E10). Fish were gutted, packed in ice and transported to the laboratory on the day of slaughter; all analyses were performed the next day. Three samples of Rainbow trout (imported) cultured in other countries were purchased from Estonian supermarkets (sample T1–Finland, T2–Norway, and T3–Finland). The imported trout had been slaughtered 4–6 days before purchase and had already been gutted, packed in plastic bag, and transported to the laboratory within an hour after purchase, where the fish was immediately packed in ice. All analyses were performed

the following day. All measurements were carried out in three repetitions.

The moisture content of the fish samples was measured using a halogen moisture analyzer (HR 83, Mettler Toledo, Switzerland). The protein content of the fish samples was measured by Kjeldahl method (Velp Scientifica UDK 142, Italy). The lipid content of fish was measured by Soxhlet method (Velp Scientifica SER 148 Solvent Extractor, Italy).

The fatty acid profile of trout samples was determined as fatty acid methyl esters (FAMEs). The Bligh & Dyer (1959) method was used for lipid extraction. The FAMEs were prepared according to the standard EVS–EN ISO 5509:2000. The prepared methyl esters samples were injected into the gas chromatograph (Agilent 7890A GC System) equipped with a flame ionization detector (FID) at a split ratio of 1:10. Helium served as the carrier gas (flow 1 ml per min). Agilent J&W GC Column HP–88 (60 m x 0.25 mm x 0.2 μm) was used for the separation of FAMEs. The analytical conditions were: injector port temperature −250°C and detector temperature −280°C. The oven was programmed from 125–230°C. Retention times of FAMEs of the standard mixture were used to identify chromatographic peaks of the sample. Supelco 37 Component FAME mix was used as standard FAME mixture. Fatty acid content in the samples was calculated, based on the peak area ratio and expressed as g fatty acid per 100 g lipid.

All necessary reagents were purchased from Sigma-Aldrich, Germany.

Statistical Analysis

XLSTAT (2010, AddInsoft, France) was used for lipid, moisture, protein, and FA ($P = 0.05$) Analysis of Variance (ANOVA) between samples. Principal Component Analysis (PCA) was used to visualize relations between samples. Statistically significant correlations (Pearson, $P = 0.05$) are given in this paper. Samples were clustered using K-means clustering according to the lipid content.

RESULTS AND DISCUSSION

Moisture, lipid and protein composition of Estonian farmed and imported Rainbow trout are shown in Table 1. The moisture content was in the range of 63.8-73.4%, the lipid content was in the range of 2.1-11.6%, and the protein ranged from 19.7-23.1%. There was a strong negative correlation between water and lipid content of the Rainbow trout ($R = -0.92$, $P = 0.05$). According to the lipid content clustering analysis (k–means) was performed, which indicated that there were three Rainbow trout groups: group 1 (G1)–lipid content 2.1–3.9%; group 2 (G2)–lipid content 4.6–7.1%; and group 3 (G3)–lipid content 9.3–11.6%.

The PCA plot (Figure 1) shows the location of the Rainbow trout in multivariate space according to the first (PC1) and second (PC2) principal component. The first and second principal components explained 98% of the total variance between the samples. The PCA plot confirms Rainbow trout grouping into three groups as the first principal component divides the samples according to their lipid contents. Sample T3 was higher in protein content than the rest of the samples.

The FA contents of Estonian farmed and imported Rainbow trout are shown in Table 2. In all samples, C16:0, C18:1, and docosahexaenoic acid (DHA, C22:6n–3) were dominant, which has also been observed by other researchers (Blanchet et al., 2005; Suzuki et al., 1986). The linoleic acid (18:2n–6) content in all analyzed Rainbow

Table 1. Moisture, lipid and protein composition (g per100 g wet meat) in Rainbow trout.

Sample	Moisture	Protein	Lipid
E1	70.3 ± 0.4	21.3 ± 0.0	3.0 ± 0.2
E2	71.1 ± 0.3	20.7 ± 0.3	4.6 ± 0.1
E3	71.1 ± 0.3	20.5 ± 0.2	4.9 ± 0.0
E4	73.4 ± 0.7	20.4 ± 0.3	2.1 ± 0.2
E5	67.6 ± 1.2	19.8 ± 0.0	5.3 ± 0.4
E6	72.4 ± 0.2	20.2 ± 0.5	3.5 ± 0.1
E7	70.3 ± 0.4	20.7 ± 0.2	5.3 ± 0.2
E8	71.8 ± 1.5	19.8 ± 0.2	3.9 ± 0.1
E9	70.0 ± 1.4	19.6 ± 0.9	5.4 ± 0.4
E10	67.6 ± 0.9	20.8 ± 0.9	7.1 ± 0.6
T1	63.8 ± 0.6	19.9 ± 0.2	11.6 ± 0.3
T2	65.2 ± 1.0	19.7 ± 0.1	9.3 ± 0.5
T3	68.2 ± 0.2	23.1 ± 0.3	5.2 ± 0.1

*Values are mean ± SE

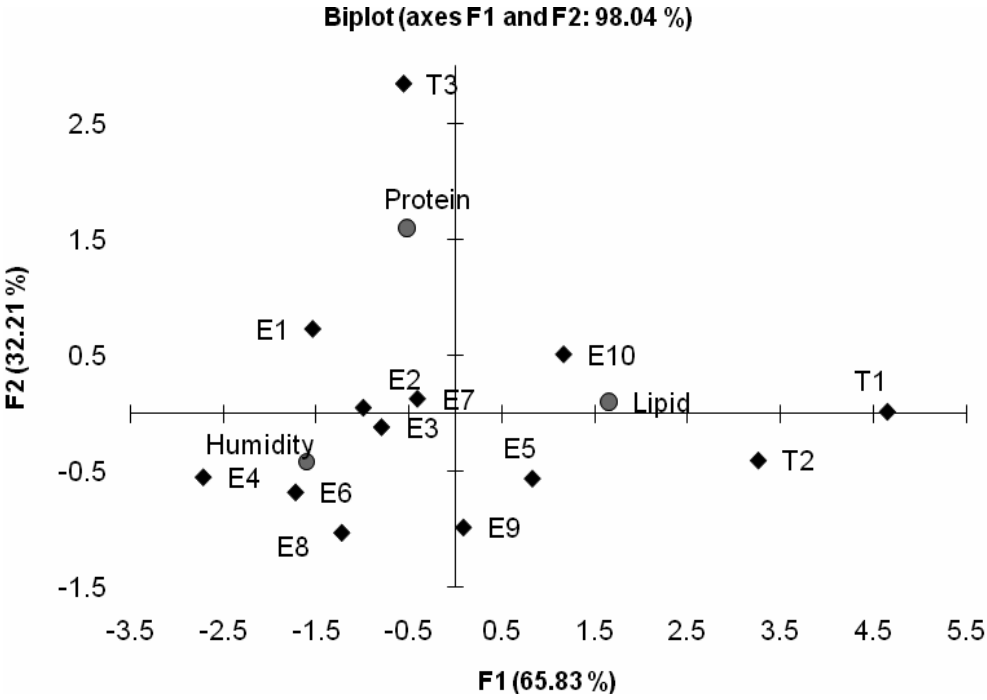

Figure 1. PCA of the moisture, lipid and protein content of Rainbow trout samples.

Table 2. Fatty acid composition (g per 100 g lipid) in Rainbow trout.

Fatty acids	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	T1	T2	T3
C14:0	4.4 ± 0.1	4.8 ± 0.1	4.2 ± 0.1	4.9 ± 0.2	4.9 ± 0.1	4.0 ± 0.1	4.5 ± 0.3	3.8 ± 0.1	5.2 ± 0.2	4.9 ± 0.1	4.2 ± 0.1	4.2 ± 0.0	5.0 ± 0.2
C16:0	17.4 ± 0.1	17.7 ± 0.3	16.0 ± 0.1	17.7 ± 0.2	14.7 ± 0.2	15.6 ± 0.2	15.1 ± 0.5	16.0 ± 0.3	16.9 ± 0.3	16.6 ± 0.1	13.8 ± 0.4	13.1 ± 0.0	15.0 ± 0.3
C18:0	3.4 ± 0.0	3.0 ± 0.1	3.1 ± 0.0	2.8 ± 0.0	2.2 ± 0.0	2.8 ± 0.1	2.7 ± 0.2	3.2 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	3.0 ± 0.1	2.8 ± 0.1	3.9 ± 1.1
C16:1	5.6 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.5 ± 0.1	5.1 ± 0.0	4.8 ± 0.0	5.3 ± 0.1	4.8 ± 0.0	5.6 ± 0.2	5.2 ± 0.1	5.4 ± 0.1	4.9 ± 0.0	5.9 ± 0.2
C18:1	23.9 ± 0.3	25.9 ± 0.4	30.8 ± 0.2	19.5 ± 0.2	27.4 ± 0.4	27.0 ± 0.2	28.9 ± 0.3	28.2 ± 0.1	26.7 ± 0.3	28.7 ± 0.1	32.6 ± 0.5	32.7 ± 0.0	23.8 ± 0.5
C20:1	2.5 ± 0.0	2.7 ± 0.0	3.1 ± 0.0	3.6 ± 0.0	3.4 ± 0.1	2.5 ± 0.0	3.0 ± 0.0	2.4 ± 0.0	3.6 ± 0.0	3.6 ± 0.0	3.3 ± 0.1	3.3 ± 0.0	6.2 ± 0.0
C18:2	7.7 ± 0.1	8.7 ± 0.1	9.7 ± 0.1	7.0 ± 0.1	9.5 ± 0.3	8.9 ± 0.1	9.6 ± 0.0	11.1 ± 0.0	8.5 ± 0.1	9.3 ± 0.1	11.6 ± 0.2	11.9 ± 0.1	9.7 ± 0.2
C18:3	2.6 ± 0.0	3.1 ± 0.0	3.5 ± 0.0	2.6 ± 0.0	3.8 ± 0.1	3.7 ± 0.0	3.9 ± 0.0	3.4 ± 0.0	3.7 ± 0.0	3.9 ± 0.0	4.4 ± 0.1	4.4 ± 0.0	2.2 ± 0.1
EPA	7.3 ± 0.1	6.8 ± 0.0	5.0 ± 0.1	5.7 ± 0.1	5.4 ± 0.2	6.1 ± 0.1	6.1 ± 0.2	6.4 ± 0.1	4.6 ± 0.1	4.2 ± 0.0	5.0 ± 0.0	4.8 ± 0.0	5.1 ± 0.1
DHA	22.4 ± 0.6	19.2 ± 0.3	16.6 ± 0.4	27.8 ± 0.7	20.7 ± 0.9	21.9 ± 0.5	18.0 ± 1.0	17.9 ± 0.3	19.1 ± 0.8	17.4 ± 0.2	13.8 ± 0.2	14.8 ± 0.1	20.6 ± 0.9
SFA	25.4 ± 0.1	25.8 ± 0.4	23.5 ± 0.1	25.6 ± 0.4	22.0 ± 0.3	22.6 ± 0.3	22.7 ± 0.9	23.3 ± 0.5	25.5 ± 0.4	25.1 ± 0.3	21.3 ± 0.6	20.5 ± 0.1	24.0 ± 0.8
MUFA	32.9 ± 0.4	34.8 ± 0.5	39.9 ± 0.3	29.6 ± 0.2	36.7 ± 0.6	35.1 ± 0.2	37.9 ± 0.3	36.2 ± 0.1	36.7 ± 0.5	38.2 ± 0.0	42.0 ± 0.6	41.6 ± 0.0	36.5 ± 0.8
PUFA	41.7 ± 0.5	39.4 ± 0.1	36.6 ± 0.3	44.8 ± 0.6	41.2 ± 0.3	42.3 ± 0.6	39.4 ± 1.3	40.5 ± 0.4	37.8 ± 0.8	36.7 ± 0.2	36.7 ± 0.2	37.9 ± 0.1	39.5 ± 0.8
n3	32.5 ± 0.6	29.3 ± 0.2	25.3 ± 0.4	36.3 ± 0.7	30.1 ± 0.6	31.9 ± 0.6	28.2 ± 1.2	27.8 ± 0.4	27.7 ± 0.9	25.7 ± 0.2	23.5 ± 0.2	24.3 ± 0.1	28.1 ± 0.9
n6	9.2 ± 0.1	10.2 ± 0.1	11.3 ± 0.1	8.5 ± 0.1	11.1 ± 0.3	10.4 ± 0.1	11.2 ± 0.1	12.8 ± 0.0	10.1 ± 0.0	11.0 ± 0.1	13.2 ± 0.2	13.6 ± 0.1	11.3 ± 0.2
n3:n6	3.6 ± 0.1	2.9 ± 0.1	2.2 ± 0.1	4.3 ± 0.1	2.7 ± 0.1	3.1 ± 0.1	2.5 ± 0.1	2.2 ± 0.0	2.7 ± 0.1	2.3 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	2.5 ± 0.1

*Values are mean ± SE. Only the major FAs (≥ 2 % and above) are listed. The other FAs are C12:0, C14:1, C18:3n–6, C20:0, C20:1, C20:2n–6, C20:3n–6, C22:0, C20:3n–3, C22:1, C20:4n–6, C22:2n–6, C24:0. The full table is available on request from the corresponding author.

trout ranged from 7.0–11.9g per 100g lipid. Linoleic acid was the major n–6 polyunsaturated fatty acid (PUFA) in Rainbow trout and is responsible for the n–3 per n–6 PUFA ratios of 1.8–3.6, which correlates well with earlier research results by Blanchett et al. (2005). According to n–3 per n–6 ratio Rainbow trout quality can be optimized, because linoleic acid comes mostly from plant–derived oils that are used in fish feed. According to Bell et al. (2001) n–3 per n–6 ratio can be improved by using feed which consists of at least two–thirds fish meal. DHA in Rainbow trout varied from 13.8 to 27.8g per 100g lipid, which is higher compared to salmon DHA values (13.115.2g per 100g lipid; Blanchett et al., 2005). Most fish species can desaturate and elongate 18:2(n–6) and 18:3(n–3) to their C20 and C22 homologues (Henderson, 1996) and this also explains strong negative correlations found in this study between DHA and 18:2n–6 (–0.85) and 18:3n–3 (–0.72), since lower PUFA diet stimulates the conversion of 18:3n–3 to DHA (Bell et al., 2001). EPA in Rainbow trout varied from 4.2 to 7.3g per 100g lipid, which is also in agreement with previous studies by Blanchett et al (2005).

The total lipid content in different Rainbow trout varied mostly more than 5.5 fold, but FA proportions were very similar in all trout. In order to evaluate possible significant differences among three Rainbow trout groups with different lipid composition, ANOVA analysis was performed on FA composition of groups, and results are shown in Table 3. Saturated fatty acid (SFA) content was similar in groups G1 and G2, but different in group G3. Monounsaturated fatty acid (MUFA) and n–3 content was significantly different in all fish groups. PUFA content was similar in groups G2 and G3, but different in group G1. Significant difference among all fish groups was noted in two dominant FAs: 18:1 and DHA.

Table 3. Fatty acid composition (g per 100 g lipid) in Rainbow trout groups G1, G2 and G3 with different lipid content

Fatty acids	G1	G2	G3
C14:0	4.3 ± 0.5 [b]	4.8 ± 0.4 [a]	4.2 ± 0.1 [b]
C16:0	16.7 ± 1.0 [a]	16.0 ± 1.1 [a]	13.4 ± 0.5 [b]
C18:0	3.1 ± 0.3 [a]	3.0 ± 0.6 [a]	2.9 ± 0.1 [a]
C16:1	5.2 ± 0.4 [a]	5.4 ± 0.3 [a]	5.1 ± 0.3 [a]
C18:1	24.7 ± 3.5 [c]	27.6 ± 2.2 [b]	32.7 ± 0.3 [a]
C20:1	2.7 ± 0.5 [b]	3.7 ± 1.1 [a]	3.3 ± 0.0 [a, b]
C18:2n–6	8.7 ± 1.6 [b]	9.3 ± 0.5 [b]	11.8 ± 0.2 [a]
C18:3n–3	3.1 ± 0.5 [b]	3.4 ± 0.6 [b]	4.4 ± 0.0 [a]
C20:5n–3 (EPA)	6.4 ± 0.6 [a]	5.3 ± 0.8 [b]	4.9 ± 0.1 [b]
C22:6n–3 (DHA)	22.5 ± 3.7 [a]	18.8 ± 1.6 [b]	14.3 ± 0.6 [c]
SFA	24.2 ± 1.4 [a]	24.1 ± 1.4 [a]	20.9 ± 0.6 [b]
MUFA	33.5 ± 2.6 [c]	37.2 ± 1.6 [b]	41.8 ± 0.4 [a]
PUFA	42.3 ± 1.7 [a]	38.7 ± 1.7 [b]	37.3 ± 0.6 [b]
n–3	32.1 ± 3.2 [a]	27.8 ± 1.8 [b]	23.9 ± 0.5 [c]
n–6	10.2 ± 1.7 [b]	10.9 ± 0.5 [b]	13.4 ± 0.2 [a]
n–3:n–6	3.3 ± 0.8 [a]	2.6 ± 0.2 [b]	1.8 ± 0.0 [c]

*Values are mean ± SE. Mean values denoted with a, b, c are significantly different in Rainbow trout groups with different lipid composition (P = 0.05).

CONCLUSIONS

High lipid content and optimum FA composition is considered to be positive criteria for the nutritional value of Rainbow trout. The total lipid content in different Rainbow trout varied more than 5.5 fold, but FA proportions were very similar in all samples. DHA and EPA, as most important essential FAs, content in all analyzed Rainbow trout was sufficient and generally higher in Estonian farmed trout than in imported trout, and correlated well with results from previous research. However, it is important to note that Estonian farmed Rainbow trout had generally lower lipid content. Because of that the amounts of essential FAs in the same size portion of fish in weight were on average 1.6 fold smaller in Estonian farmed Rainbow trout.

Estonian Rainbow trout smolts come from the same hatcheries and are fed the same commercial feed as Rainbow trout farmed in other countries. The difference in Estonian Rainbow trout lipid content is mainly influenced by environment, particularly temperature: optimum is from 8 to 15ºC, but in Estonia there are long periods where fish do not feed (cold winters and hot summers). Environmental influence needs to be compensated by proper feeding regimes and a longer growth period. In order to maintain and raise the quality and stability of Estonian farmed Rainbow trout it is vital for Estonian fish farmers to unify the level of lipid content of their fish.

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